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2014 FEED DEALERS SEMINARS

Table of Contents

	Page
Impacts and Evaluation of Subclinical Hypocalcemia in Dairy Cattle (Manuscript) <i>B.M. Sweeney, E.M. Martens, M.J. Felipe, and T.R. Overton</i>	1
The Impacts and Evaluation of Subclinical Hypocalcemia (PowerPoint Slides) <i>B.M. Sweeney, E.M. Martens, M.J. Felipe, and T.R. Overton</i>	9
Research Update: Starch Level and Rumensin in Fresh Cow Rations <i>M.M. McCarthy, T. Yasui, C.M. Ryan, G.D. Mechor, and T.R. Overton</i>	27
Strategies to Improve Fresh Cow Energy Metabolism <i>M.M. McCarthy and T.R. Overton</i>	37
Phosphorus Nutrition of Dairy Cattle – Update <i>L.E. Chase and C.M. Ryan</i>	55
Factors Affecting Milk Components <i>T.R. Overton</i>	61
Shredlage – What Have We Learned? <i>L.E. Chase</i>	81

IMPACTS AND EVALUATION OF SUBCLINICAL HYPOCALCEMIA IN DAIRY CATTLE

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The initiation of lactation in dairy cattle requires a coordinated increase in supply of calcium to the mammary gland as part of the homeorhetic shift from supporting the demands of pregnancy to those of lactation (Bauman and Currie, 1980). The increase in release of calcium stored in bone, increase in absorption of calcium from the diet, and decrease in excretion of calcium at the kidney must provide at least a two-fold increase in supply of calcium for colostrum production compared to that required by the growing fetus within a very short time frame (House and Bell, 1993; Goff and Horst, 1997). A large proportion of dairy cows are unable to meet this challenge in the 48 hours after parturition and therefore have an acute drop in blood calcium (hypocalcemia). Whereas clinical hypocalcemia (blood calcium typically below 5.0 mg/dL) incidence can be reduced to 5% or less, the incidence of subclinical hypocalcemia (SCH; blood calcium <8.0 mg/dL) has been shown to be 47% in cows entering their second or greater lactation, and 25% in first lactation animals (Reinhardt et al., 2011).

IMPACT OF SUBCLINICAL HYPOCALCEMIA

Clinical hypocalcemia is recognized as a risk factor for several other negative health events including dystocia, retained placenta, ketosis and mastitis (Curtis et al., 1983). Cows with clinical hypocalcemia have also been shown to produce less milk in early lactation (Rajala-Schultz et al., 1999). Recent research has shown similar negative associations between SCH and poor energy status, infectious disease, and productive and reproductive outcomes.

Cows with SCH have been shown to have higher plasma non-esterified fatty acids prepartum (NEFA), higher β -hydroxybutyrate (BHBA) postpartum and an increase in liver lipid accumulation at 7 and 35 days postpartum indicating that cows with low blood calcium have poorer energy status and excessive body fat mobilization (Martinez et al., 2012; Chamberlin et al., 2013). High prepartum NEFA and postpartum BHBA have been associated with losses in milk production (Ospina et al., 2010). Chapinal et al. (2012) showed that cows in herds with $\geq 35\%$ incidence of SCH in the week postpartum had increased odds of displaced abomasum as well as a 3.8 kg/d reduction in milk production at the first test day. These data indicate that SCH exacerbates the negative energy balance experienced by the transition cow, resulting in increased susceptibility to other disorders as well as decreased productivity.

Calcium is an essential component for activation of many different cells throughout the body. A crucial role of calcium in the early lactation dairy cow is activation of the immune cells, primarily neutrophils, that are responsible for clearing the uterus of infectious agents after parturition. Kimura (2006) showed that immune cells of hypocalcemic cows had reduced calcium release from intracellular stores upon activation, causing a blunted response and furthering the immunosuppression experienced around the time of parturition (Kehrli et al., 1989). Martinez et al. (2012) also showed an association between hypocalcemia and reduced neutrophil function, as well as an increased risk for uterine disease in hypocalcemic cows compared to cows with normal blood calcium. Ultimately, hypocalcemic cows in this study tended to have delayed pregnancy. At the herd-level, a study conducted on commercial herds found that with higher incidence of SCH in the week postpartum, there was a 30% reduction in pregnancy at first artificial insemination, which occurred in 40% of herds sampled (Chapinal et al., 2012).

Subclinical hypocalcemia has serious implications in regards to the future health of the cow, as well as important economical consequences as a result of reduced milk production and poorer reproductive performance. Strategies for preventing milk fever, such as feeding a diet low or negative in dietary cation anion difference prepartum, are widely implemented, however there is a lack of evidence supporting these strategies as effective in reducing incidence of SCH. The ability to assess the calcium status of fresh cows on the farm will be an important tool to move forward in managing for lower incidence of SCH by providing a means for assessing prevention strategies as well as identification of cows in need of intervention.

EVALUATION OF SUBCLINICAL HYPOCALCEMIA

Due to the lack of clinical symptoms associated with SCH, the disease can only be identified through the measurement of blood calcium. Blood calcium is present in three forms; ionized calcium and calcium bound to either proteins or anions (Rosol et al., 1995). It is hypothesized that ionized calcium should be a more accurate reflection of the functional calcium status of the cow because this is the form of calcium available to cells to perform intracellular signaling, necessary for such functions as contraction of muscles or activation of immune cells (Kimura et al., 2006).

Traditionally, the cutpoint used to identify SCH has been 8.0 mg/dL (2.0 mmol/L) total calcium, and 4.0 mg/dL (1.0 mmol/L) ionized calcium due to an assumption that total blood calcium is composed of 50% ionized calcium. This relationship is assumed to be fairly stable and therefore any variation caused by bound calcium when measuring total calcium is thought to be negligible. Some evidence suggests that the relationship between total and ionized calcium may be different around the time of parturition due to the rapid increase in mobilization of bone calcium and increased absorption from the diet (Ballantine and Herbein, 1991).

Ionized calcium is seldom used as a measure of calcium status due to challenges with accurate measurement. Exposure of samples to air as well as the use of

anticoagulants changes the amount of ionized calcium in the sample (Boinke et al., 1991). The iSTAT Portable Clinical Analyzer was identified as a tool for accurate measurement of ionized calcium due to the ability to analyze whole blood within minutes of sample collection and without the use of anticoagulants. The on-farm application of this tool is unlikely due to the cost of analysis but the accurate ionized calcium measurement allows for characterization of the ionized versus total calcium relationship.

Blood total calcium is typically measured to assess the calcium status of a cow due to ease of sample handling, storage and analysis. In research, samples are often sent to a diagnostic laboratory for analysis. The value of this method for on farm decision making is minimal due to the long turnaround time for results. Although potentially effective treatment options for SCH are available (Oetzel and Miller, 2012), methods for identifying cows affected with SCH within the time relevant for treatment are not widely implemented. A tool for measuring blood total calcium, the IDEXX VetTest Chemistry Analyzer, was identified as having potential as an on-farm tool because of its ease of use and relatively lower cost of analysis compared to the diagnostic lab.

A study was conducted in our laboratory to assess options for measuring calcium status of cows in the immediate postpartum period. The objectives of the study were to assess the interrelationships between blood minerals measured by the IDEXX VetTest and the reference method, as well as the relationship between ionized and total calcium measured in early postpartum dairy cows, and their relative values as predictors of neutrophil oxidative burst activity.

EXPERIMENTAL APPROACH

Blood samples were taken from multiparous Holstein cows (n=33) twice in the 24 hours after calving and once daily through 5 days in milk. If cows became clinically hypocalcemia, only samples taken before treatment with intravenous calcium were included in the data set. All blood samples were analyzed for ionized calcium within 3 minutes of sample collection using the iSTAT Portable Clinical Analyzer (iSTAT; Abbot Point of Care, Inc., Princeton, NJ), which measures ionized calcium with an ion selective electrode. The sample was then centrifuged and the serum harvested for analysis of total calcium, magnesium and phosphorous on the IDEXX VetTest Chemistry Analyzer (VetTest; IDEXX Laboratories, Inc., Westbrook, ME), the remaining serum was sent to the Michigan State Diagnostic Center for Population and Animal Health (DC) for a full mineral panel analysis, both of which use a colorimetric method for determining total calcium concentration. An additional blood sample was taken between 2 and 5 days in milk for flow cytometric determination of neutrophil oxidative burst activity using a commercial kit (Phagoburst, Glycotope GmbH, Berlin, Germany).

Data were analyzed using the statistical software SAS version 9.2 (Cary, NC). Correlations between mineral measurements were determined using PROC CORR. Cutpoints to diagnose SCH (defined as diagnostic lab total calcium ≤ 8.0 mg/dL) using ionized calcium and total calcium (measured by the VetTest) providing the highest

combined specificity and sensitivity were determined using the receiver operator characteristic. To determine the relative value of ionized versus total calcium as predictors of neutrophil oxidative burst activity, a multivariate model was created using PROC GLM in which the following variables were considered; parity (2nd lactation vs. 3rd lactation and greater), calving ease (scale 1-5), block (summer 2013 vs. winter 2014), presence or absence of disease prior to neutrophil assessment (diseases included fever, retained placenta, displaced abomasum, ketosis, mastitis, and were diagnosed by the herd health staff), as well as biologically relevant 2-way interactions. Three models were created; a base model containing only variables significant at a p-value of less than 0.10, the base model plus the minimum total blood calcium, and the base model plus the minimum ionized blood calcium. Models containing calcium parameters were compared to the base model using a Chi-Square test and the ionized calcium model was compared to the total calcium model using the R² value.

PERFORMANCE OF A POTENTIAL ON-FARM DIAGNOSTIC TOOL

Measurement of blood minerals by the VetTest were highly correlated with measurements by the DC. Magnesium ($r=0.92$, slope=0.94, $p<0.0001$) and phosphorous ($r=0.95$, slope=0.92, $p<0.0001$) measurements were highly correlated with minimal bias. Measurements of blood total calcium were highly correlated ($r=0.95$, $p<0.0001$), however, a slope of 0.82 indicated slight bias by the VetTest for higher measurements of blood calcium, with greater bias as the calcium content of the sample increased (Figure 1). Using a cutpoint of 8.9 mg/dL, SCH could be diagnosed with a sensitivity and specificity of 89% and 89%, respectively. This indicates that with an adjusted cutpoint, the VetTest has potential to be used on-farm as a tool for reliably diagnosing SCH.

RELATIONSHIP BETWEEN IONIZED AND TOTAL CALCIUM POSTPARTUM

When all samples collected throughout the 5 day sampling period were considered, the correlation between ionized calcium, measured by the iSTAT, and total calcium, measured by the DC, was strong ($r=0.93$) and the slope indicated that ionized calcium constituted 53% of total calcium. However, this relationship varied throughout the first 5 DIM, with 57% of total calcium being ionized in samples taken in the 24 hours after calving (Figure 2). The cutpoint that diagnosed SCH with the highest combined sensitivity and specificity (95% and 79%, respectively) was 4.68 mg/dL, which differed from the assumed 4.0 mg/dL. This indicates that the dynamics between ionized calcium and total calcium in the day following calving may differ from a cow not experiencing such dramatic changes in calcium metabolism. Using cutpoints for ionized calcium that are based on assumptions of the relationship between ionized and total calcium may not be identifying the same cows as using total calcium for diagnosis of SCH.

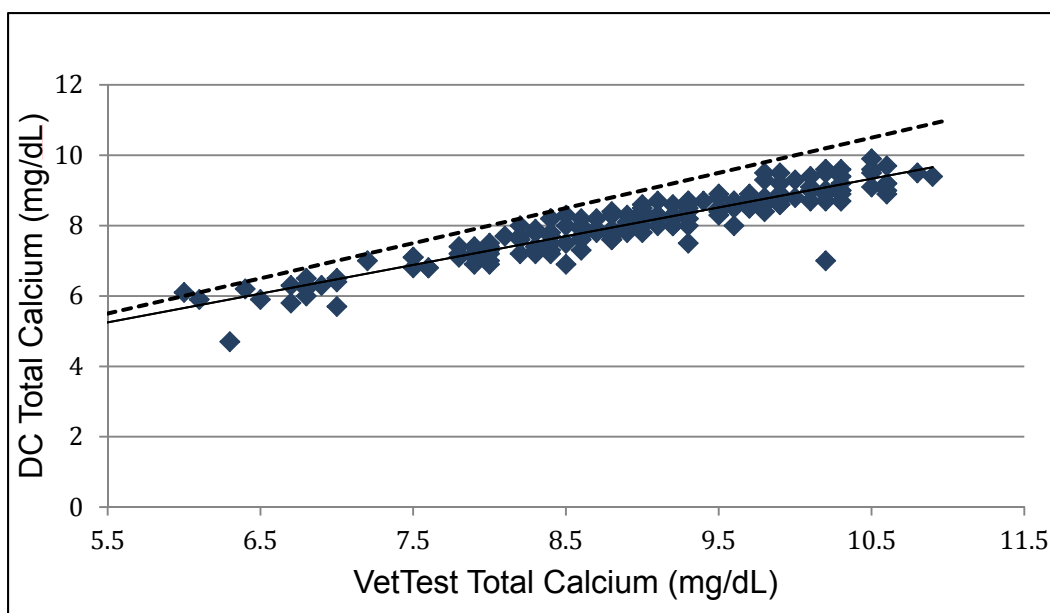


Figure 1. Relationship between total blood calcium measured by the IDEXX VetTest versus the Michigan State Diagnostic Center for Population and Animal Health. The dashed line represents unity.

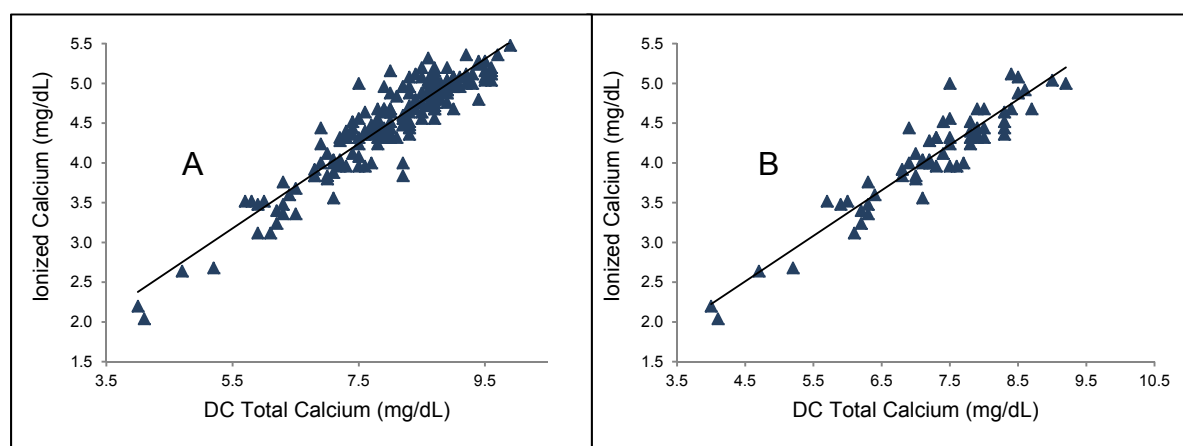


Figure 2. The relationship between ionized calcium and total calcium from samples taken throughout the 5 days after calving (A) and samples taken in the 24 hours after calving (B).

IONIZED VS. TOTAL CALCIUM AS INDICATORS OF CALCIUM STATUS

The base model for explaining neutrophil oxidative burst activity contained parity, block and presence or absence of disease as explanatory variables. The model was significantly improved when either minimum total calcium ($p < 0.05$) or minimum ionized calcium ($p < 0.0025$) were added to the model, indicating that calcium status is an important factor in determining the ability of neutrophils to mount an oxidative burst response. The R^2 values for the models containing minimum ionized calcium and minimum total calcium were 0.61 and 0.52, respectively. In both models, the calcium

parameter had a significant interaction with diseases status such that cows which had one or more disease prior to measurement of neutrophil function had greater oxidative burst reaction as blood calcium content increased (Figure 3), whereas cows with no disease had no change in oxidative burst response as blood calcium increased. Considering that the model containing minimum total calcium provided the same information as the model containing ionized calcium, the added complication and cost of measuring ionized calcium does not seem to justify slight improvements in explaining this functional outcome related to calcium status.

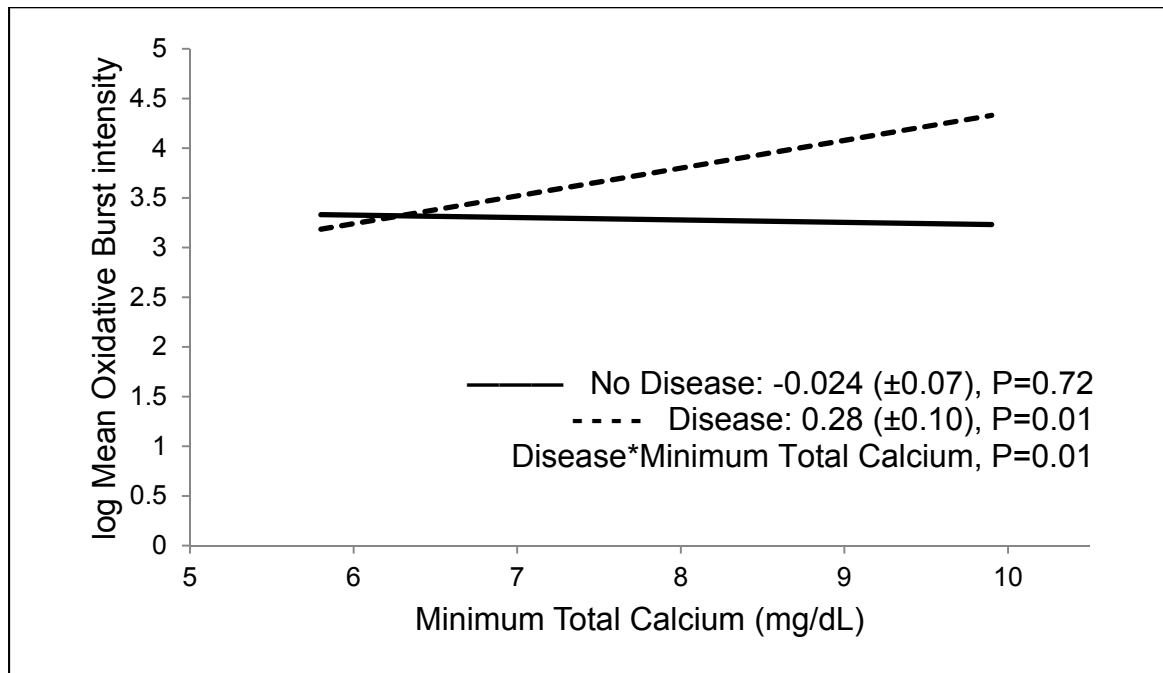


Figure 3. The interaction between disease status and minimum blood total calcium concentration in impacting the neutrophil oxidative burst response. Slope (\pm SE) is shown for each disease category.

CONCLUSIONS

The relationship between ionized and total calcium in the immediate postpartum period may differ from the assumed relationship between the parameters, this may cause some discrepancies for diagnosis of SCH depending on the calcium parameter used. Ionized calcium does not show a strong advantage over total calcium as a predictor of neutrophil oxidative burst activity, suggesting that total calcium should be sufficient when measuring the calcium status of early postpartum cows. The IDEXX VetTest presents an opportunity for on farm determination of calcium status, which can allow for rapid diagnosis of SCH for treatment decisions as well as determination of SCH incidence on the farm. Measuring the calcium status of the fresh cow is the first step in making intervention or management decisions in order to decrease the long-term consequences of SCH on a herd.

ACKNOWLEDGEMENTS

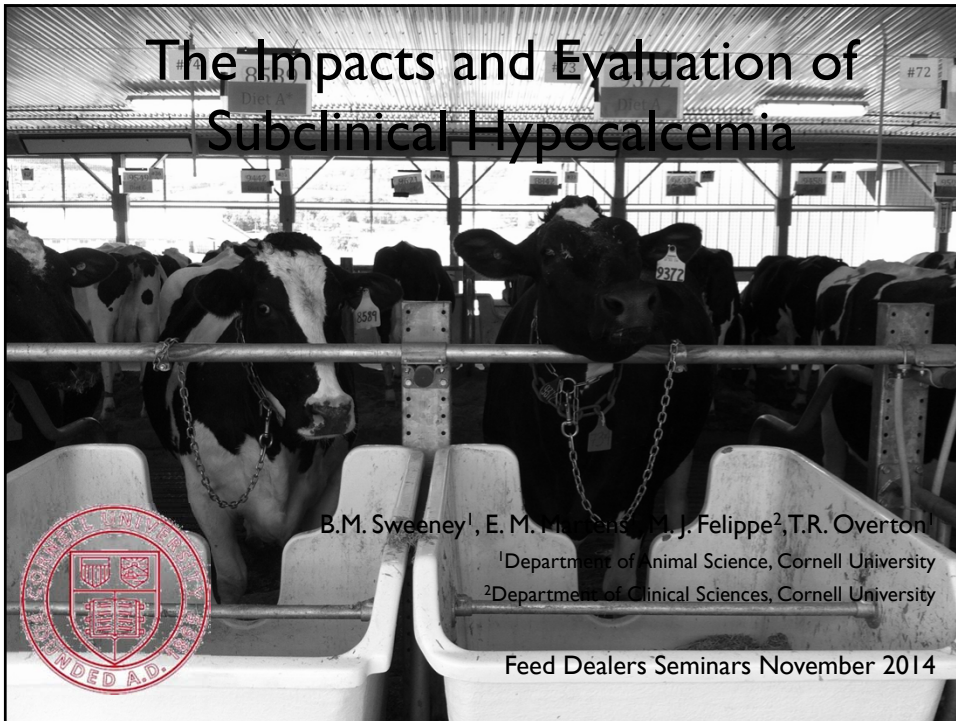
We would like to acknowledge Dr. Ken Zanzalari, Prince Agri-Products Inc., and Dr. John Lawrence, IDEXX Laboratories, Inc., for their contributions to the conduct of this work.

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The Impacts and Evaluation of Subclinical Hypocalcemia



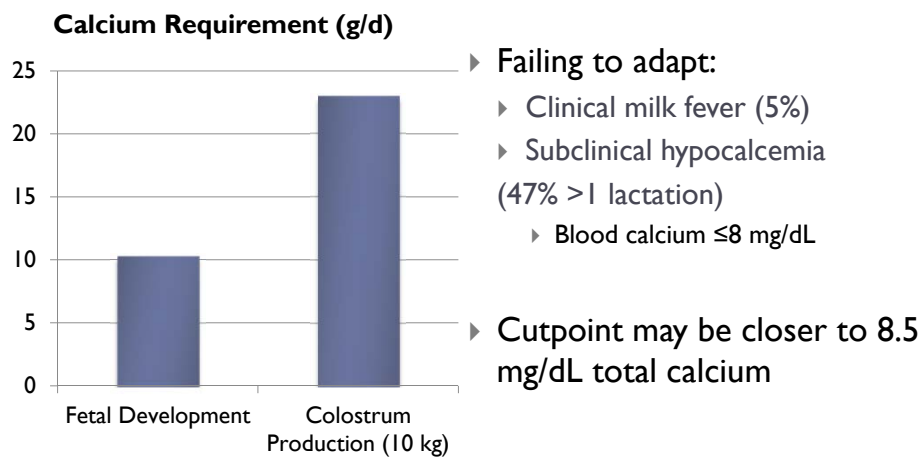
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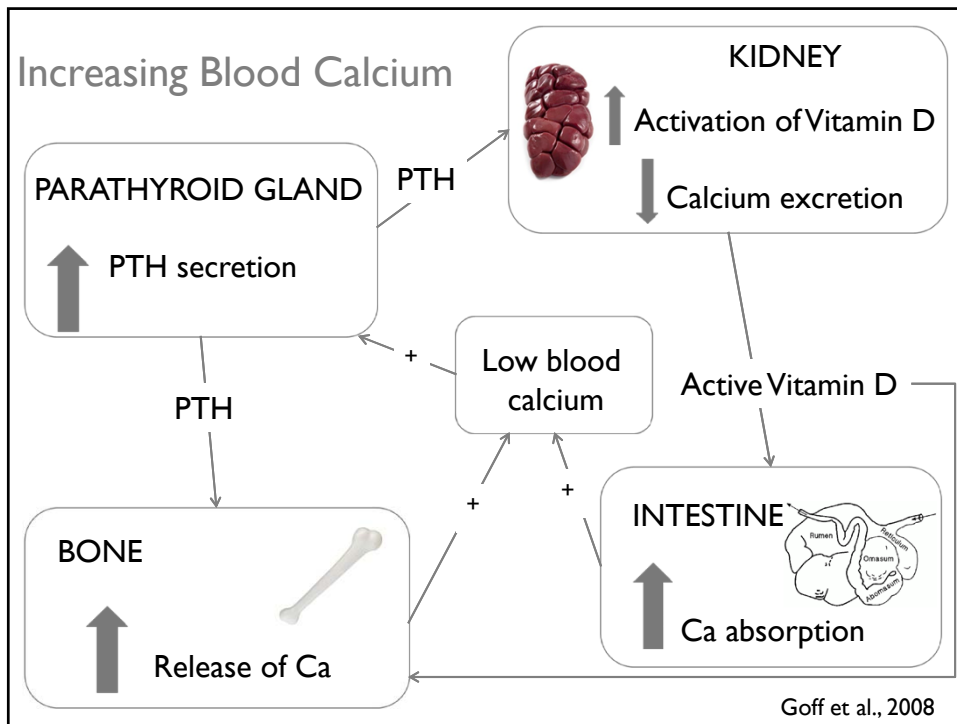
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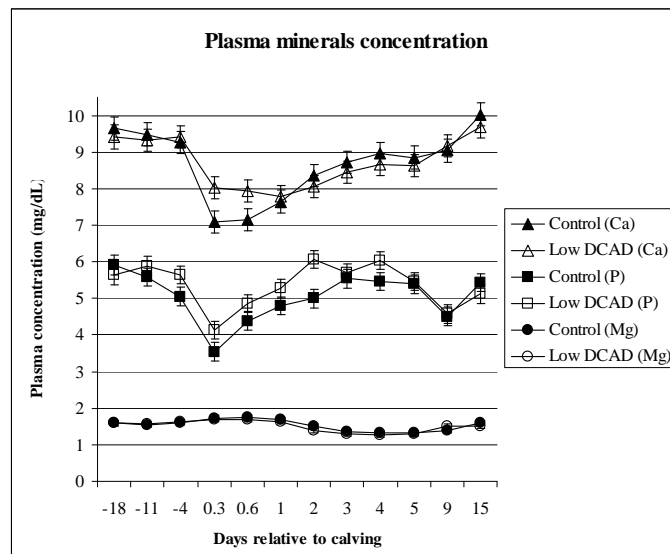
Calcium Demand Around Calving



House and Bell, 1993, Goff et al., 1997, Reinhardt et al., 2011



Mineral status

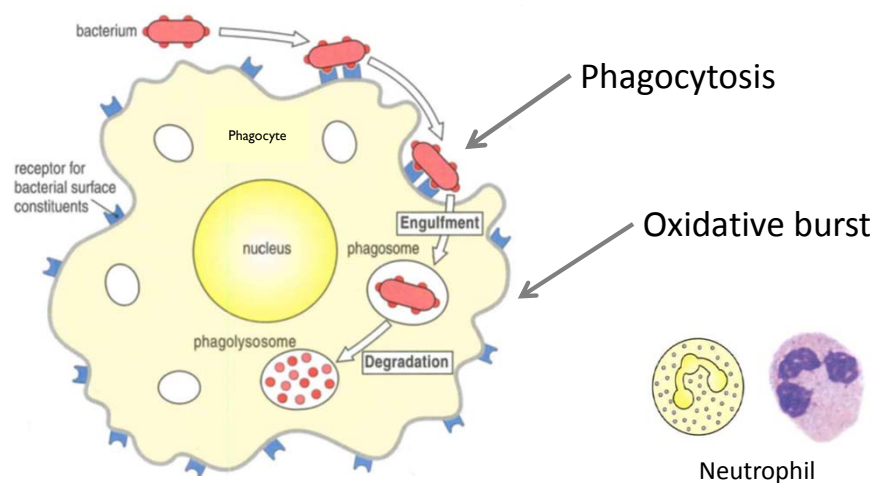


Ramos Nieves et al., 2009

Health Consequences

- ▶ Clinical hypocalcemia (milk fever):
 - ▶ ↑ risk of retained placenta, dystocia, ketosis, mastitis (Curtis et al., 1983)
- ▶ Subclinical hypocalcemia (SCH):
 - ▶ Compromised
 - ▶ immune function
 - ▶ metabolic health
 - ▶ productive and reproductive performance

Immune Function: Focus on Neutrophils

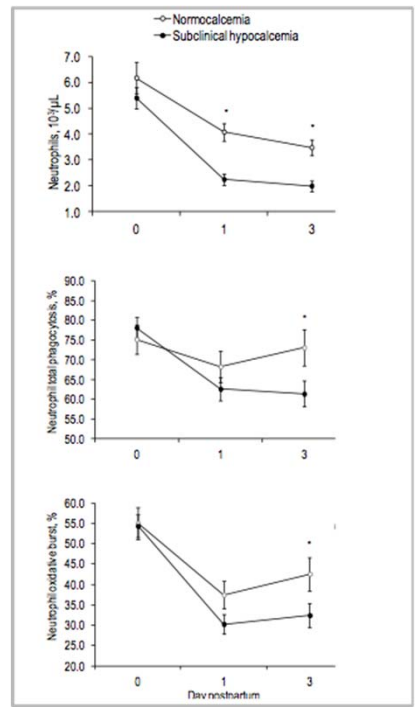


Figures from Parham, P. and C. Janeway, 2009. The Immune System. Garland Science, London; New York

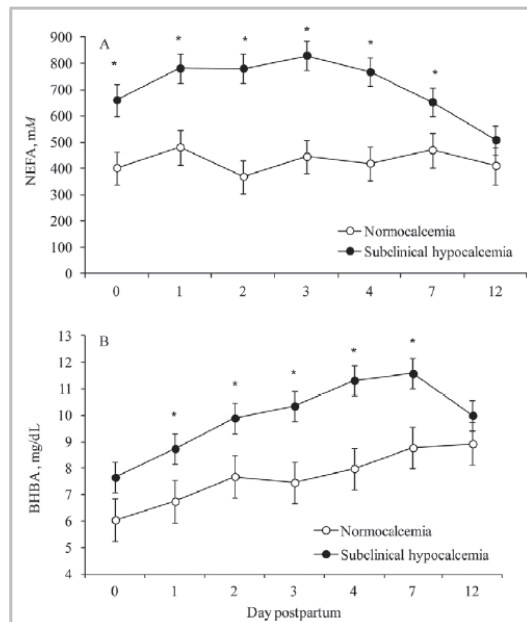
Cows with SCH have further compromised immune function

- ▶ ↓ signaling capacity of immune cells (Kimura et al., 2006)
- ▶ ↓ killing ability of neutrophils
- ▶ 3.24X more likely to have metritis
- ▶ 1.8X more likely to have cytological endometritis (subclinical endometritis)

Martinez et al. 2012

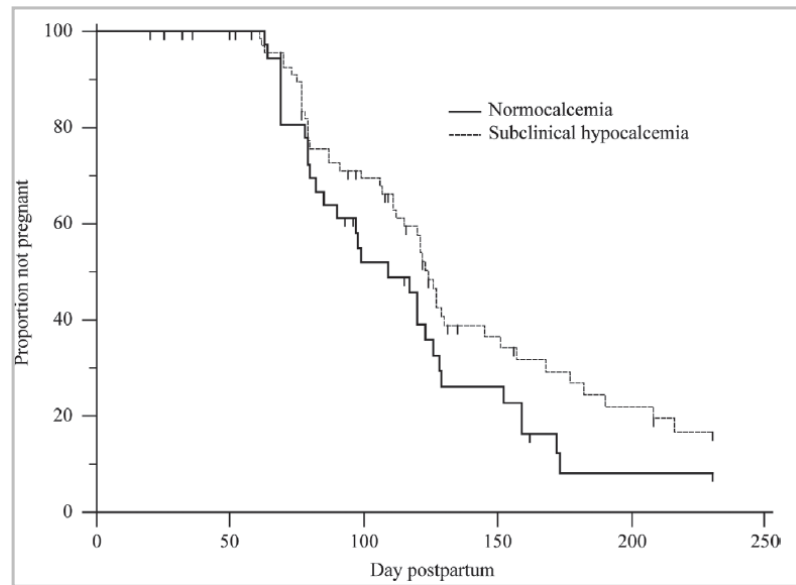


Cows with SCH have higher NEFA and BHBA



Martinez et al., 2012

Cows with SCH have delayed reproduction



Martinez et al., 2012

Herd-level associations of low Ca during wk +1 (<8.4 mg/dL) with outcomes

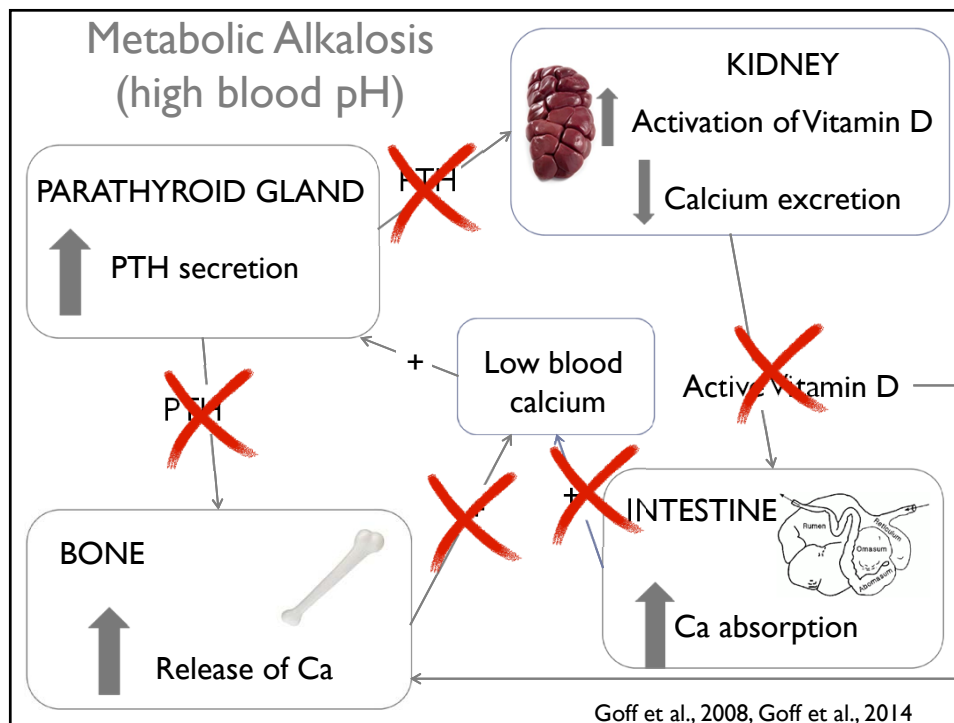
Item	Herd-level threshold (%)	Farms above threshold (%)	Outcome	P-value
DA (all cows)	≥ 35	24	OR = 2.4	0.003
DA (multiparous)	≥ 30	43	OR = 1.9	0.004
Milk ¹ (all cows)	≥ 15	73	- 3.8 kg/d	0.01
Milk (multiparous)	≥ 25	55	- 2.9 kg/d	0.05
Pregnancy 1 st AI (all cows)	≥ 25	40	OR = 0.7	0.02

¹ At 1st DHI test day

Chapinal et al., 2012

How do we manage SCH?

- Prevention?
 - Close-up dry nutrition strategies
 - DCAD
 - Fresh cow nutrition strategies
 - Mg and Ca levels?
- Monitoring
 - Herd level and cow level monitoring



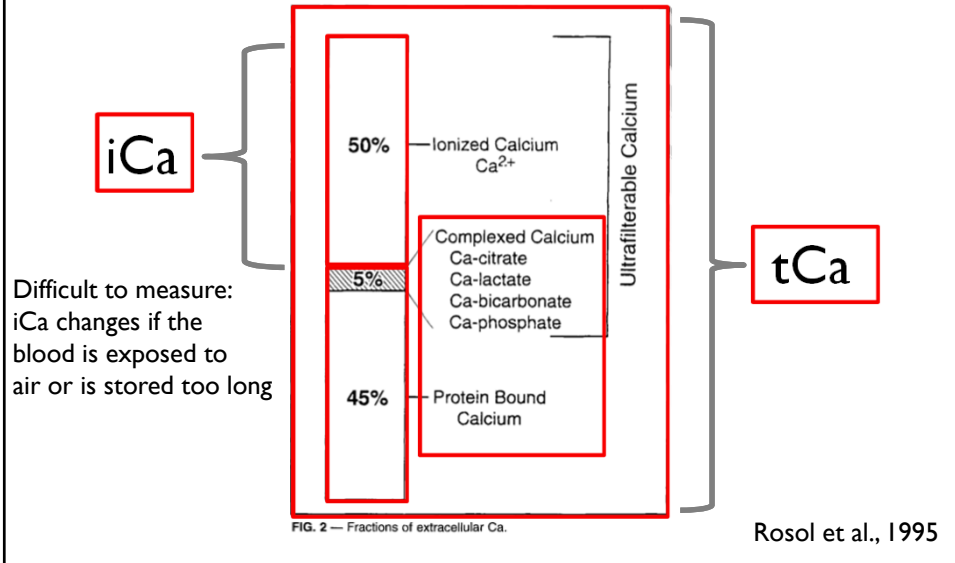
Strategies for application of DCAD for close-up dry cows

- Focus on feeding low K (and Na) forages and feeds to close-up dry cows
 - *Calculated DCAD ~ +10 mEq/100 g of DM*
 - *Urine pH ~ 8.3 to 8.5*
- Feeding low K forages along with partial use of anionic supplement in close-up ration or one-group dry cow ration
 - *Calculated DCAD ~ 0 mEq/100 g of DM*
 - *Urine pH ~ 7.5*
- Feeding low K forages along with full use of anionic supplement in close-up ration or one-group dry cow ration
 - *Calculated DCAD ~ -10 to -15 mEq/100 g of DM*
 - *Urine pH ~ 6.0 to 7.0 – need to monitor weekly and adjust DCAD*
- Need to supplement Mg (dietary target 0.40 to 0.45%) during close-up
- Recommend supplementing Ca (0.9 to 1.0% if low K only; 1.4 to 1.5% if full anionic diet)

Measuring Ca Status in Fresh Cows

- ▶ **Herd Level Monitoring**
 - ▶ Determine herd incidence of SCH
 - ▶ Use as a “barometer” for early detection of issues
 - ▶ Measure efficacy of prevention strategies
- ▶ **Cow Level Monitoring**
 - ▶ Identify cows in need of treatment

Calcium Distribution in the Blood



Utilization of iCa Results

- ▶ What is the correct cutpoint?
 - ▶ Assume that iCa is 50% of tCa
 - ▶ May differ due to increase Ca metabolism postpartum
 - ▶ Typically 4.0 mg/dL is used as a cutpoint for SCH
- ▶ Lacking data to associate cutpoint of iCa with health outcomes

Ballantine and Herbein, 1991

Determination of Cutpoints

- ▶ 8.4 mg/dL tCa (wk +1 postcalving)
 - ▶ Associated with risk of decreased production, increased risk of DA, decreased odds of pregnancy at first AI (Chapinal et al., 2012)
- ▶ 8.59 mg/dL tCa (during 3 days postcalving)
 - ▶ Based on ability to predict metritis (Martinez et al., 2012)

On Farm Utilization

- ▶ Diagnosis of SCH on-farm is of increasing interest due to:
 - ▶ Associations with negative health/performance
 - ▶ Treatment options
- ▶ No clinical signs – must measure Ca in blood!

Methods for Measuring Ca Status Postpartum

- Ionized or total calcium?
- Serum/plasma or whole blood?
- On-farm or in a lab?
- Tool/method?

Method	Diagnostic Lab (DL)	IDEXX VetTest (VetTest)	iSTAT Portable Analyzer (iSTAT)
Ca Fraction Measured	tCa	tCa	iCa
Use	In a lab Herd Level Monitoring Slow Result Turnaround	On-farm Herd & Cow Level Monitoring Fast Results Easy to use	On-farm Herd & Cow Level Monitoring Fast Results and sound method for iCa Difficult to use
Cost	Not economical for regular monitoring	Potentially economical	Expensive!



On-Farm Use of the VetTest

- ▶ Collect blood sample 8-16 hrs postcalving
- ▶ Centrifuge
- ▶ Insert slides for analytes to measure (ie. tCa, Mg, P)
- ▶ Follow on screen instructions to put sample in machine
- ▶ Results in 6 min

- ▶ Total time ~30 min



Objectives

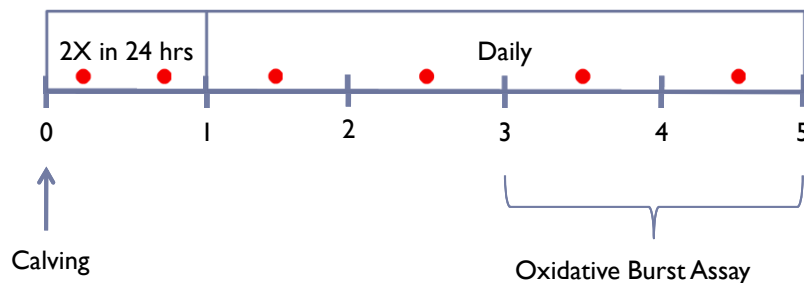
- ▶ tCa DL vs. tCa VetTest
 - ▶ Relationship between two methods of measuring the same component
 - ▶ Agreement for diagnosis of SCH

- ▶ iCa vs. tCa
 - ▶ Relationship between these two Ca fractions postpartum
 - ▶ Agreement for diagnosis of SCH
 - ▶ Relative value as predictors of neutrophil oxidative burst activity

Sweeney et al. 2014. 97:(Suppl.1;Abstr.)

Materials and Methods

- ▶ 34 multiparous cows
- ▶ Blood sampling scheme:



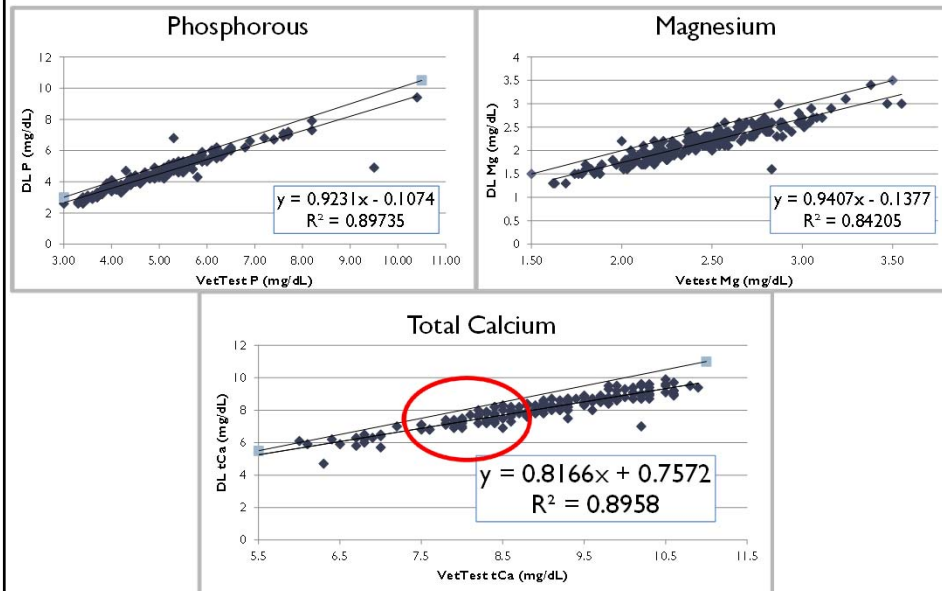
Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

Materials and Methods

- ▶ Blood collected via syringe containing no anticoagulant
- ▶ Dispensed into iSTAT cartridge within 1 minute of collection
- ▶ Remainder of blood centrifuged and tested on the VetTest (within 2 hours of collection)
- ▶ Serum stored at -20°C and sent to Michigan State University Diagnostic Lab
- ▶ Neutrophil oxidative burst capacity was determined using a commercial kit (PhagoBURST, Glycotope, Heidelberg, Germany)

Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

Serum Minerals: DL vs. VetTest



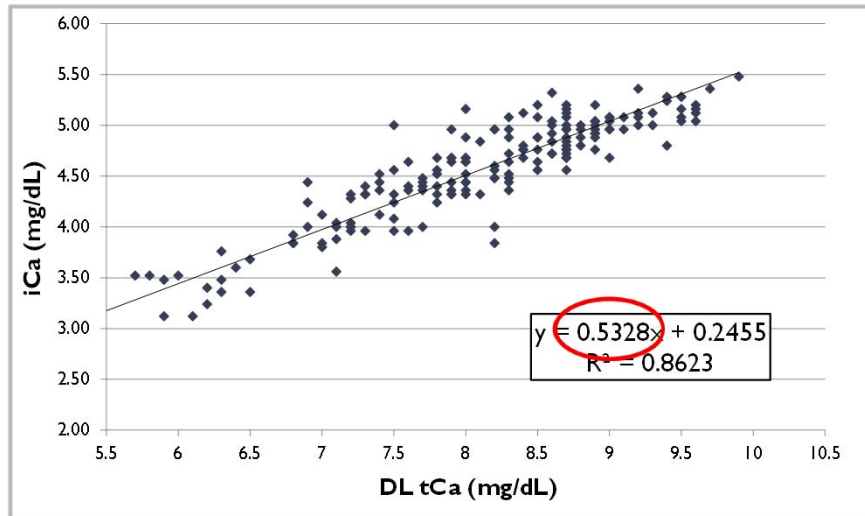
Sweeney et al. 2014. 97:(Suppl.1;Abstr.)

VetTest tCa Cutpoint Determination

- ▶ Using a cutpoint of 8.9 mg/dL for VetTest tCa, we can reach the same diagnosis as the DL (using 8.0 mg/dL cutpoint)
 - ▶ Sensitivity: 89%
 - ▶ Specificity: 89%
 - ▶ AUC: 0.953

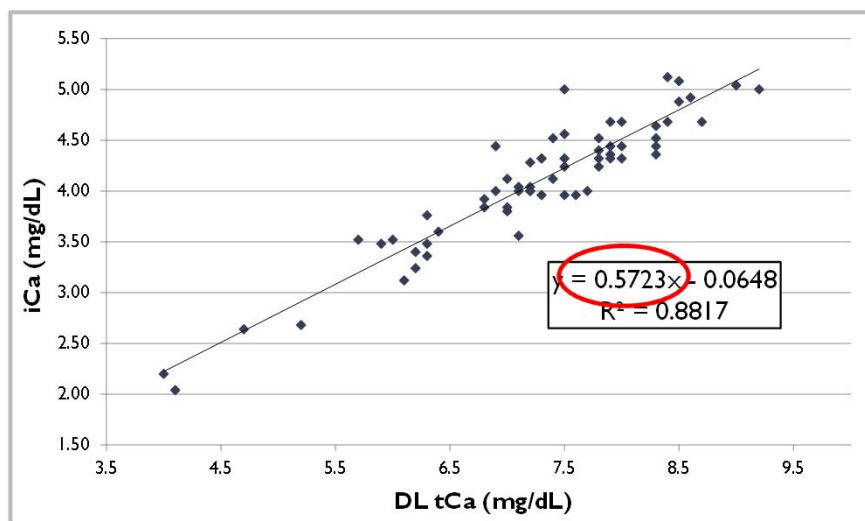
Sweeney et al. 2014. 97:(Suppl.1;Abstr.)

All Samples DL tCa vs. iCa



Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

Samples in first 24 hours DL tCa vs. iCa



Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

iCa Cutpoint Determination

- ▶ Using a cutpoint of 4.68 mg/dL for iCa, we can reach the same diagnosis as the DL (using 8.0 mg/dL cutpoint)
 - ▶ Sensitivity: 95%
 - ▶ Specificity: 79%
 - ▶ AUC: 0.915

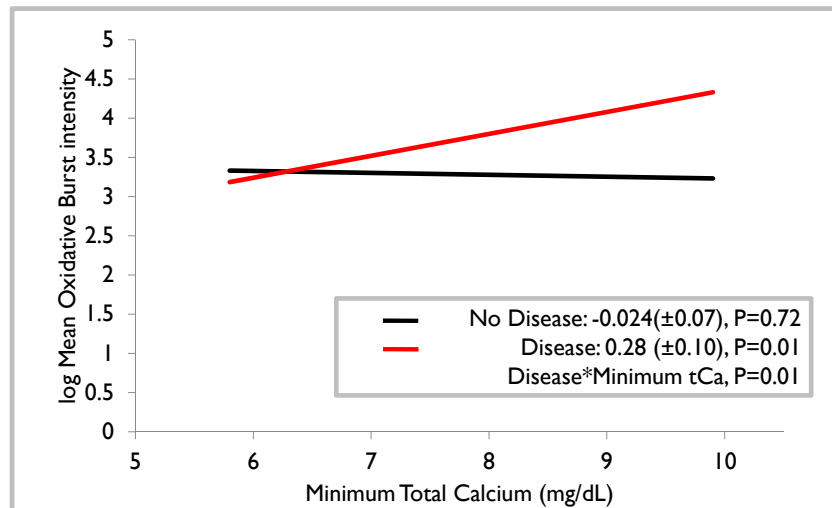
Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

Models Explaining *E. coli* Stimulated Neutrophil Oxidative Burst Intensity

Model	R ²	Comparison to Base Model (P-value)
Base Model (parity, disease, block)	0.385	
Base Model + Minimum tCa	0.515	<0.05
Base Model + Minimum iCa	0.612	<0.0025

Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

Minimum tCa interaction with disease status



Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

Conclusions

- ▶ More work needed before iCa can be used alone for determination of SCH
 - ▶ Actual proportion of iCa (as a percent of tCa)
 - ▶ Proper cutpoint using iCa
 - ▶ iCa may be a better reflection of what is happening in the cow, but is difficult to measure
 - ▶ SCH can be reliably diagnosed using the VetTest, but a different cutpoint is necessary
- ***Potential for on-farm tool

Sweeney et al. 2014. 97:(Suppl. I; Abstr.)

Take Home Message

- ▶ SCH is affecting productive and reproductive efficiency
 - ▶ Continue to explore efficacy of prevention/treatment strategies
- ▶ Measuring Ca in fresh cows gives us the ability to approach SCH diagnostically at the herd and cow level
 - ▶ tCa is probably the most practical
 - ▶ We do have some options for on-farm assessment
 - ▶ Herd-level monitoring may be a valuable tool

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Questions?



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RESEARCH UPDATE: STARCH LEVEL AND RUMENSIN IN FRESH COW RATIOS

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INTRODUCTION

Many studies have evaluated the effect of prepartum nutrition on postpartum lactation performance and its associated metabolic changes. Surprisingly there have been relatively few studies that have evaluated the effects of postpartum nutrition on these metabolic adaptations and their effects on production performance.

After parturition the nutrients required for milk synthesis utilize a large portion of maternal nutrients (Bauman and Currie, 1980). In the immediate postpartum period dry matter intake (DMI) is insufficient to support the high milk production of early lactation and results in increased mobilization of adipose tissue and the oxidation of non-esterified fatty acids (NEFA) by the liver. Higher DMI generally results in lower circulating NEFA and has been associated with improved health and performance (Ingvarsen and Andersen, 2000).

Optimizing DMI during this postpartum period is especially important to provide sufficient energy to support milk production. Due to the increased glucose demand for milk lactose synthesis, liver glucose production nearly doubles within 11 days of calving compared to prepartum glucose output (Reynolds et al., 2003). Propionate that is produced via fermentation of starch in the rumen is the main precursor for liver glucose production. Rumensin also has been shown to increase ruminal propionate production (Armentano and Young, 1983). While there is a large increase in the liver's utilization of lactate, glycerol, and the glucogenic amino acids postpartum, propionate is still quantitatively the greatest contributor to liver gluconeogenesis at about 60% of precursor supply (Reynolds et al., 2003). Because of this increased demand for glucose postpartum, the liver should have the capacity to direct any additional propionate supply towards glucose synthesis during this early postpartum period (Drackley et al., 2001).

Allen et al. (2009) proposed that liver energy status is a major regulator of DMI in dairy cows. The premise is that when oxidative fuel metabolism (mainly propionate, but also NEFA) by the liver exceeds energy requirements, the brain is signaled to reduce DMI. This hepatic oxidation theory would suggest that feeding diets that would increase propionate supply (e.g. greater amounts or fermentability of starch, addition of Rumensin) during early lactation would decrease DMI via this liver signaling mechanism. If the hepatic oxidation theory applies in this manner to the early lactation period, then reducing the dietary starch content during this period would likely increase DMI by reducing propionate production in the rumen and decreasing the hypophagic effect from propionate oxidation (Allen et al., 2009). However, because liver energy requirements increase dramatically at the onset of lactation (Reynolds et al., 2003) and adipose mobilization is increased (Vernon, 2005), we believe that NEFA are likely to be

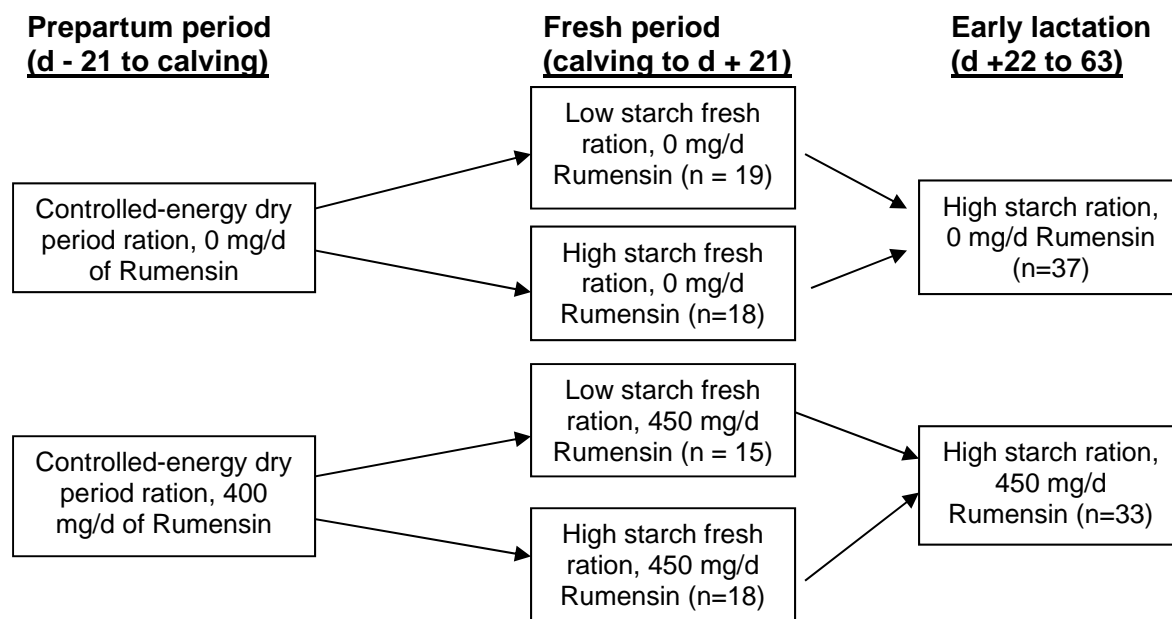
the predominant oxidative fuel for the liver. Recent work from the Allen lab with early lactation animals has shown propionate infusion to be more hypophagic in animals with higher liver acetyl CoA concentrations, which is indicative of higher NEFA mobilization (Stocks and Allen, 2012; 2013). We believe any hypophagic effect of propionate is likely to be reduced immediately after calving because of the large increases in liver energy demands postpartum (Overton, 2011), although, the effect of propionate load in early lactation is still in debate.

The objectives of this research were to further investigate these effects of propiogenic diets on intake, production, energy metabolism, and liver propionate metabolism in early lactation dairy cows.

EXPERIMENTAL APPROACH

A total of 70 Holstein cows (n= 49 multiparous and n=21 primiparous) were enrolled in this experiment at 21 d before expected calving and fed a controlled energy diet based upon corn silage, wheat straw, and a concentrate mix and topdressed daily with a pellet containing either 0 or 400 mg/d of Rumensin. After calving, cows were further randomized within Rumensin treatment and fed either a 26.2% starch (HS) or 21.5% starch (LS) fresh cow diet with Rumensin administration continuing at either 0 or 450 mg/d by daily topdress pellet (Figure 1). From d 22 through 63 all cows were fed the HS diet with their assigned daily Rumensin topdress.

Figure 1. Experimental design treatment schematic.



The postpartum diets were formulated on the basis of a lactation diet in which BMR corn silage was the predominant forage, with smaller amounts of wheat straw and haylage (Table 1). The concentrate portion of the HS diet was based on ground corn grain (20.1% of diet DM). For the LS diet corn grain (9.8% of diet DM) was partially

replaced with citrus pulp (6.5% of diet DM) and soy hulls (3.4% of diet DM). The HS and LS diets were formulated to contain 28.0 and 21.0% starch, respectively, although, the analyzed starch content of the HS and LS diets were 26.2 and 21.5%. The analyzed starch content of the HS diet was lower than expected; however, the difference between the two diets is still large enough that it makes for a meaningful comparison.

Table 1. Ingredient and nutrient content of experimental diets (DM basis)

Item	Prepartum	Postpartum	
		High Starch	Low Starch
Ingredient, % of DM			
Corn silage	39.5	—	—
BMR corn silage	—	37.0	37.0
Haylage	—	9.3	9.3
Wheat straw	20.5	11.1	11.1
Corn grain	3.8	20.1	9.8
Corn germ meal	—	2.3	5.4
Citrus pulp	6.6	0.8	6.5
Soy hulls	6.6	—	3.4
Soybean meal	5.0	5.5	3.6
Canola meal	4.3	2.5	2.0
Blood meal	1.0	1.9	1.9
Minerals and vitamins ¹	6.6	5.3	5.8
Topdress	6.1	4.2	4.2
Chemical Analysis			
DM, %	50.7 ± 2.4	48.3 ± 2.7	48.0 ± 3.2
CP, %	13.0 ± 0.8	15.5 ± 1.2	15.4 ± 0.8
ADF, %	28.2 ± 1.2	22.7 ± 1.2	25.2 ± 1.2
NDF, %	42.9 ± 2.0	34.3 ± 1.5	36.9 ± 1.5
30 h NDFD, % of NDF	—	55.1 ± 2.0	56.1 ± 1.4
Sugar, %	4.9 ± 0.8	3.5 ± 0.6	4.5 ± 0.4
Starch, %	17.4 ± 1.2	26.2 ± 1.2	21.5 ± 1.0
Fat, %	2.6 ± 0.2	4.0 ± 0.2	2.2 ± 0.6
Calcium, %	1.28 ± 0.16	0.94 ± 0.09	1.01 ± 0.04
Phosphorous, %	0.30 ± 0.02	0.34 ± 0.02	0.34 ± 0.02
Magnesium, %	0.41 ± 0.04	0.28 ± 0.02	0.3 ± 0.03
Potassium, %	1.12 ± 0.13	1.12 ± 0.09	1.18 ± 0.08
Sulfur, %	0.37 ± 0.04	0.21 ± 0.09	0.22 ± 0.01
Sodium, %	0.12 ± 0.02	0.47 ± 0.08	0.46 ± 0.05
Chloride, %	0.37 ± 0.01	0.44 ± 0.04	0.44 ± 0.03

¹Contained 30,317 mg/kg of Cu, 136,466 mg/kg of Mn, 3,393 mg/kg of Co, 3,040 mg/kg of I, and 153,916 mg/kg of Zn, 30,464 IU/kg of Vitamin A, 5,862 IU/kg of Vitamin D, and 93,784 IU/kg of Vitamin E, 510,750 IU/kg of Vitamin E.

Samples of all TMR and ration ingredients were obtained weekly and composited at 4 wk intervals for analysis of chemical composition using wet chemistry techniques. All cows were weighed once weekly and body condition scores were assigned for all

cows weekly by 2 technicians using the 5 point system (Wildman et al., 1982). All cows were milked 2 times daily for the 63 d of the lactation phase of the trial and daily milk yield was measured electronically. Milk samples were collected weekly from 2 consecutive milkings obtained over a 24-h period. Samples were analyzed for milk fat, protein, and lactose.. Liver biopsies were taken on d 7 postpartum and used for an in vitro gluconeogenesis experiment.

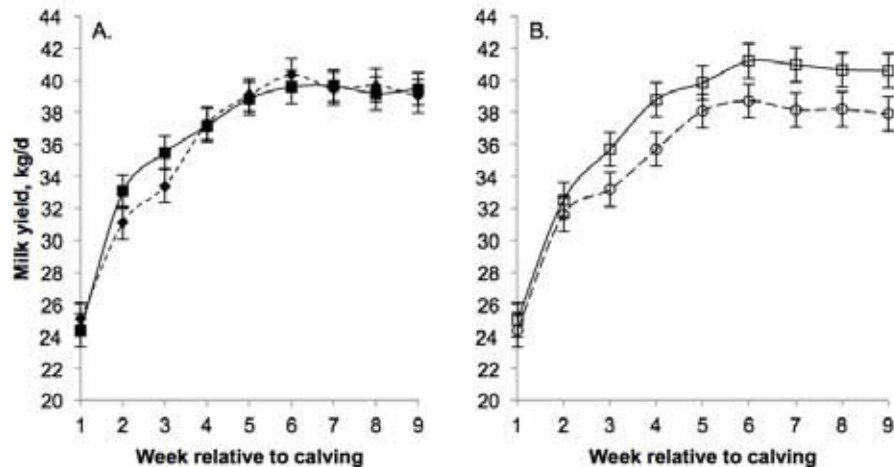
Statistical computations were performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC). Postpartum data were analyzed as a completely randomized design with a 2 × 2 factorial arrangement of treatments. Fixed effects included starch level, Rumensin treatment, parity, time (wk or d), and all 2-way interactions. The random effect was cow nested within starch and Rumensin treatment. Postpartum data were analyzed separately as wk 1 to 3 and wk 1 to 9. Data measured over time were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1996). For variables with measurements repeated over time, three covariance structures were tested: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance structure that resulted in the Akaike information criterion closest to zero was used (Littell et al., 1996). Data not analyzed over time were subjected to ANOVA using the MIXED procedure of SAS (Littell et al., 1996).

Degrees of freedom were estimated by using the Kenward-Roger option in the model statement. Least squares means for treatment effects were separated by use of the PDIF statement when the overall F-test was $P < 0.05$ and trends were declared at $0.05 < P < 0.10$. Because a subset of animals were used for the liver biopsy data trends for these data were declared at $0.05 < P < 0.15$. There was no interaction of starch × Rumensin treatment so all results will be presented as main effects of either starch or Rumensin.

DO PROPIOGENIC FRESH RATIONS AFFECT PERFORMANCE?

Milk yield data for the fresh period treatments are presented in Figure 2. The overall effect of starch level in the fresh period diet on milk yield from wk 1 through 9 was not significant ($P = 0.81$; average 30.4 kg/d); however, cows fed the HS diet had a faster increases in milk in wk 2 and 3 postpartum compared to cows fed the LS diet ($P = 0.0003$). Further evaluation of the patterns of milk yield during wk 1 to 3 using daily milk yield data suggested that cows fed the HS diet tended ($P = 0.10$) to have higher overall milk yield compared to cows fed the LS diet (31.1 vs. 29.2 kg/d). Cows fed Rumensin during the transition period produced 2.2 kg/d ($P = 0.05$) more milk than control cows when evaluated from wk 1 to 9 postpartum. Trends for Rumensin × week interactions during wk 1 to 3 for both milk yield ($P = 0.07$) and lactose yield ($P = 0.06$) suggested that yields of each were increased by wk 3 of lactation compared to control cows.

Figure 2. Least squares means and standard errors for milk yield for starch and Rumensin dietary treatments. Panel A depicts milk yields for cows fed either high (-■-) or low starch (--♦--) fresh diets. Panel B depicts milk yields for cows fed Rumensin at 0 mg/d (-○-) or 450 mg/d (-□-) during the transition period.

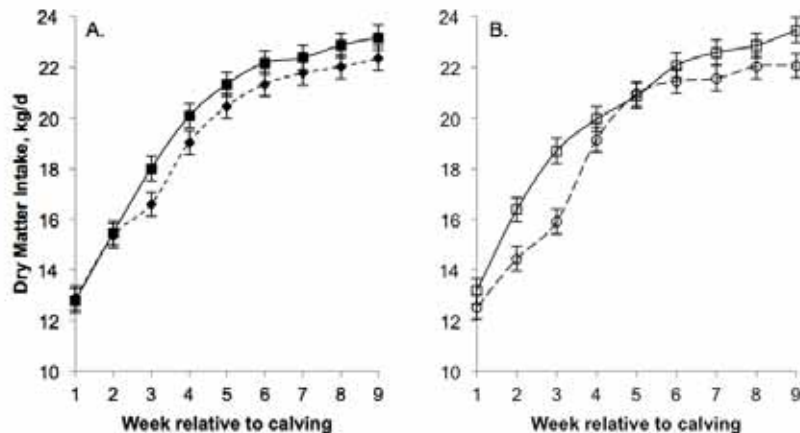


Cows fed HS fresh diets had lower percentages of milk fat (4.38 vs. 5.01%; $P = 0.01$) and true protein (3.31 vs. 3.84%; $P = 0.05$) than cows fed LS diets during wk 1 to 3 postpartum, however, when evaluated over the 9 wk postpartum period these effects were not significant. Percentages of lactose (4.60 vs. 4.83%; $P = 0.05$), and total solids (13.31 vs. 14.76%; $P = 0.009$) also were decreased during wk 1 to 3 in cows fed the HS fresh diets compared to those fed LS diets; these effects also were significant when evaluated over the 9 wk postpartum period ($P = 0.03$ for both variables). Despite the differences in milk component percentages, the effects of starch level on overall yields of milk fat, true protein, lactose, total solids, and ECM were not significant when evaluated for either wk 1 to 3 or 1 to 9 (Table 2). For cows fed the LS diet, component yields were generally higher during wk 1 compared to cows fed the LS diet, but both groups had similar component yields during wk 2 and 3. LS cows likely had greater component yields in wk 1 postpartum because they had greater adipose tissue mobilization. There was no effect of Rumensin treatment on percentages of milk true protein and total solids or yields of fat, true protein, lactose, total solids, and ECM during wk 1 to 3. During wk 1 to 9, cows fed Rumensin had lower percentages of milk lactose (4.82 vs. 4.93%; $P = 0.03$); however, there was no difference in lactose yield (average 1.76 kg/d) and percentages and yields of other milk components and ECM were not affected by treatment. Cows fed Rumensin had higher MUN during both wk 1 to 3 and wk 1 to 9 ($P = 0.007$ and $P = 0.02$, respectively).

Dry matter intake data for the fresh period treatments are presented in Figure 3. Cows fed the HS fresh diet had similar overall DMI compared to cows on the LS diet but increased ($P = 0.006$ DMI when expressed as a percentage of BW (2.67 vs. 2.41%); during wk 1 to 3. Significant interactions of starch level and week for DMI suggested that cows fed HS had a faster increase in intake ($P = 0.04$). Cows fed Rumensin had higher DMI than controls during both wk 1 to 3 (16.1 vs. 14.3 kg/d; $P = 0.004$) and wk 1

to 9 (20.0 vs. 18.9 kg/d; $P = 0.02$). There was an interaction of Rumensin \times week for both wk 1 to 3 ($P = 0.009$) and wk 1 to 9 ($P < 0.0001$) such that cows fed Rumensin had greater DMI during wk 2 and 3 postcalving.

Figure 3. Least squares means and standard errors for dry matter intake (DMI) for starch and Rumensin dietary treatments. Panel A depicts DMI for cows fed either high (—■—) or low starch (---◆---) fresh diets. Panel B depicts DMI for fed Rumensin at 0 mg/d (—●—) or 450 mg/d (—□—) during the transition period.



There was no effect of dietary starch level on postpartum BW, BW change, or average BCS; however, heifers fed the HS fresh diet lost less BCS during the first 3 wk postcalving than animals in the other treatment groups (starch \times parity interaction; $P = 0.01$). Milk production efficiency during both wk 1 to 3 and wk 1 to 9, calculated either as milk yield per unit of DMI or ECM yield per unit of DMI, was increased in cows fed LS fresh diets (Table 2). This increased milk efficiency is likely because cows fed the LS diet had decreased DMI and mobilized more adipose tissue during wk 1 to 3. Although overall effects of Rumensin treatment on postpartum BCS and BCS change during both wk 1 to 3 and 1 to 9 were not significant, an interaction of Rumensin and parity existed during wk 1 to 9 such that heifers fed Rumensin lost slightly less BCS and cows fed Rumensin lost slightly more BCS ($P = 0.006$). Overall effects of Rumensin treatment on feed efficiency, expressed as units of milk per unit of DMI, were not significant during either wk 1 to 3 or wk 1 to 9. However, when milk production efficiency was expressed as units of ECM per unit of DMI, cows fed Rumensin had slightly lower efficiency during wk 1 to 3 ($P = 0.05$), likely contributed to by the higher DMI for cows fed Rumensin.

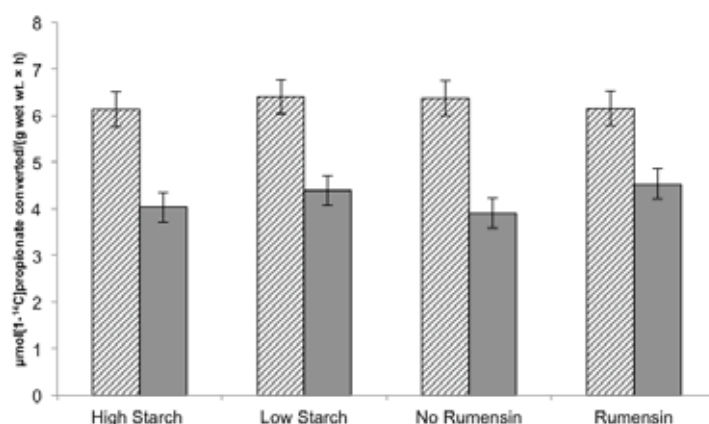
DO PROPIOGENIC DIETS AFFECT LIVER GLUCONEOGENIC CAPACITY?

Overall, animals fed diets with greater propiogenic capacity had faster increases in milk production and DMI, which would indicate that increased propionate supply in early lactation allows animals a better start. One of our main questions in conducting this study was how the liver handles propionate load in early lactation, which we evaluated

using an in vitro system by incubating liver tissue slices obtained on d 7 postpartum with a [1-¹⁴C]propionate and measuring label incorporation into glucose and CO₂.

There was no effect of starch or Rumensin treatment on liver capacity to oxidize [1-¹⁴C]propionate to CO₂ and no effects of starch on liver capacity to convert propionate to glucose (Figure 4). Cows that were fed Rumensin tended ($P = 0.14$) to have greater capacity to convert [1-¹⁴C]propionate to glucose than control cows. Heifers had greater capacity to both oxidize [1-¹⁴C]propionate to CO₂ and convert [1-¹⁴C]propionate to glucose than did multiparous animals ($P = 0.04$ and $P = 0.01$, respectively).

Figure 4. Conversion rates of [1-¹⁴C]propionate to CO₂ (striped bars) and glucose (solid bars) at d 7 postpartum for all cows (treatment indicated on the x axis).

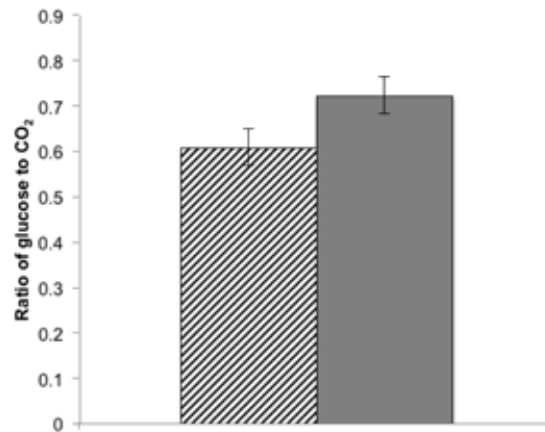


In the TCA cycle the [1-¹⁴C]propionate label randomizes such that every mole of [1-¹⁴C]propionate directed toward phosphoenolpyruvate and gluconeogenesis would yield 0.5 moles of radiolabeled CO₂ and 0.5 moles of radiolabeled glucose (Knapp et al., 1992). Therefore, the ratio of rates of conversion of radiolabeled propionate to glucose and CO₂ provides an index of the efficiency of propionate utilization for gluconeogenesis. Rumensin administration increased the ratio of glucose to CO₂ (Figure 3; $P = 0.05$), which indicates that cows fed Rumensin have a greater propensity to convert propionate to glucose (Figure 5).

IMPLICATIONS AND CONCLUSIONS

In early lactation the liver has the ability to preferentially use additional propionate supply for gluconeogenesis. Increasing the ruminal propionate supply that is available to the liver for glucose synthesis after calving provides the cow with better energy status and allows a better start to lactation. This is indicated by improvements in DMI, increased milk yield, as well as faster increases in milk yield postpartum. In cows fed more propiogenic diets the increased liver glucose output provides the animal with better energy status and less dependence on adipose mobilization. Based on the data from this study it would appear that DMI in fresh cows is not limited by propionate oxidation at the liver, and feeding diets with higher starch levels and containing Rumensin in fresh rations results in improved milk production and energetic status.

Figure 5. Ratio of glucose to CO₂ for control cows (striped bar) and for Rumensin treated cows (solid bar) at d 7 postpartum.



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Table 2. Milk yield, intake, and feed efficiency for cows fed varying levels of starch and Rumenin in the fresh period

Item	Diet ¹		SEM		Topdress ²		SEM		P-values ³						
	HS	LS	SEM	Con	Rum	Topdress ²	SEM	S	Rum	P	SxRum	SxWk	RumxWk	SxP	RumxP
Milk yield, kg/d															
wk 1 to 3	31.0	29.8	0.9	29.8	31.0		0.9	0.35	0.32	<0.001	0.45	0.002	0.07	0.57	0.63
wk 1 to 9	36.3	36.0	0.8	35.1	37.3		0.8	0.81	0.05	<0.001	0.43	0.0003	0.19	0.33	0.71
ECM, kg/d ⁵															
wk 1 to 3	34.7	36.7	1.2	35.9	35.4		1.2	0.24	0.80	<0.001	0.97	0.01	0.93	0.45	0.93
wk 1 to 9	36.9	37.6	1.0	36.8	37.8		1.0	0.59	0.47	<0.001	0.51	0.19	0.48	0.44	0.55
Fat, kg															
wk 1 to 3	1.32	1.44	0.06	1.41	1.34		0.06	0.14	0.34	<0.001	0.91	0.11	0.97	0.67	0.86
wk 1 to 9	1.33	1.37	0.04	1.35	1.35		0.05	0.48	0.93	<0.001	0.95	0.20	0.61	0.56	0.96
True protein, kg															
wk 1 to 3	0.99	1.07	0.05	1.02	1.04		0.05	0.25	0.85	<0.001	0.79	0.09	0.44	0.35	0.81
wk 1 to 9	1.03	1.06	0.03	1.02	1.06		0.04	0.53	0.33	<0.001	0.76	0.15	0.64	0.62	0.33
Lactose, kg															
wk 1 to 3	1.43	1.44	0.05	1.42	1.44		0.05	0.89	0.77	<0.001	0.58	0.04	0.06	0.23	0.90
wk 1 to 9	1.75	1.78	0.04	1.73	1.79		0.04	0.62	0.32	<0.001	0.35	0.09	0.36	0.08	0.70
Total Solids, kg															
wk 1 to 3	4.04	4.27	0.15	4.17	4.13		0.15	0.26	0.84	<0.001	0.90	0.02	0.69	0.39	0.94
wk 1 to 9	4.44	4.55	0.11	4.44	4.55		0.12	0.49	0.51	<0.001	0.44	0.21	0.63	0.26	0.49
MUN, mg/dL															
wk 1 to 3	11.6	10.9	0.4	10.5	11.9		0.4	0.16	0.007	0.05	0.86	0.44	0.46	0.89	0.59
wk 1 to 9	11.7	11.5	0.4	11.0	12.2		0.4	0.59	0.02	0.23	0.42	0.21	0.37	0.98	0.49
DMI, kg/d															
wk 1 to 3	15.6	14.8	0.4	14.3	16.1		0.4	0.21	0.004	<0.001	0.77	0.04	0.009	0.24	0.74
wk 1 to 9	19.8	19.1	0.3	18.9	20.0		0.3	0.13	0.02	<0.001	0.66	0.32	<0.001	0.99	0.67
DMI, % of BW															
wk 1 to 3	2.67	2.41	0.06	2.48	2.60		0.06	0.006	0.20	<0.001	0.71	0.01	0.29	0.84	0.25
wk 1 to 9	3.37	3.22	0.05	3.24	3.35		0.05	0.03	0.11	<0.001	0.47	<0.001	0.66	0.98	0.99
Feed efficiency															
Milk/DMI															
wk 1 to 3	1.95	2.10	0.05	2.05	2.03		0.06	0.04	0.59	0.84	0.48	0.51	0.95	0.30	0.04
wk 1 to 9	1.84	1.95	0.03	1.88	1.90		0.03	0.04	0.67	0.97	0.17	0.53	0.95	0.28	0.21
ECM/DMI															
wk 1 to 3	2.20	2.50	0.07	2.45	2.26		0.07	0.002	0.05	0.47	0.98	0.66	0.76	0.45	0.07
wk 1 to 9	1.90	2.05	0.04	1.99	1.97		0.04	0.006	0.69	0.14	0.26	0.13	0.58	0.28	0.65

¹Postpartum diets HS = High starch, LS = Low starch.

²Con = control topdress, contained 0 g/metric ton Rumenin, and Rum = Rumenin topdress, contained 441 g/metric ton Rumenin.

³S = starch level, and P = parity.

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Strategies to improve fresh cow energy metabolism

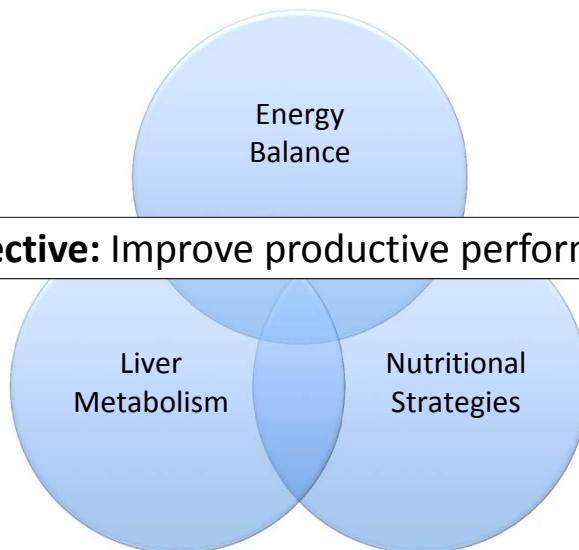
M. M. McCarthy and T. R. Overton



Cornell University

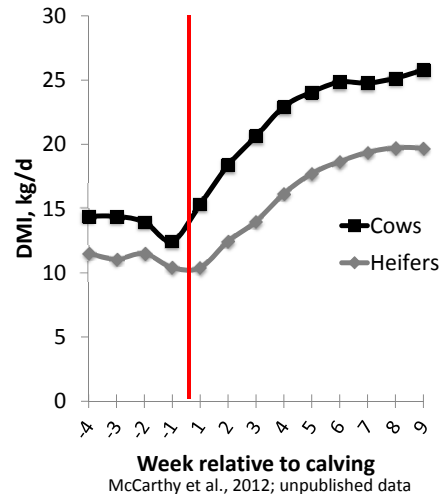
Fresh Cow

Objective: Improve productive performance



Dry Matter Intake of Early Lactation

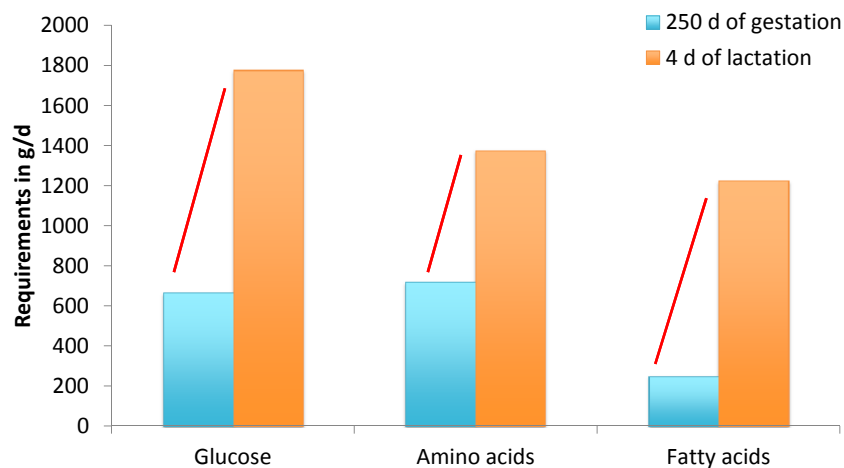
- Post calving milk production greatly increases cow's energy needs
- Dry matter intake (DMI) after calving is insufficient to meet energy demands of milk production



Bell, 1995; Ingvarsen and Andersen, 2000

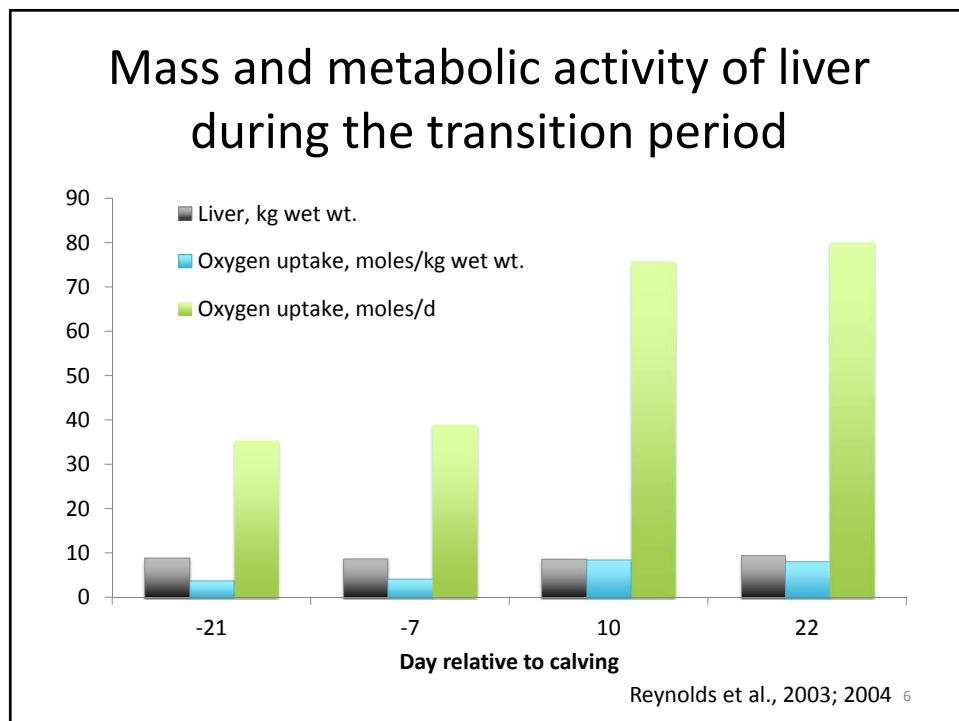
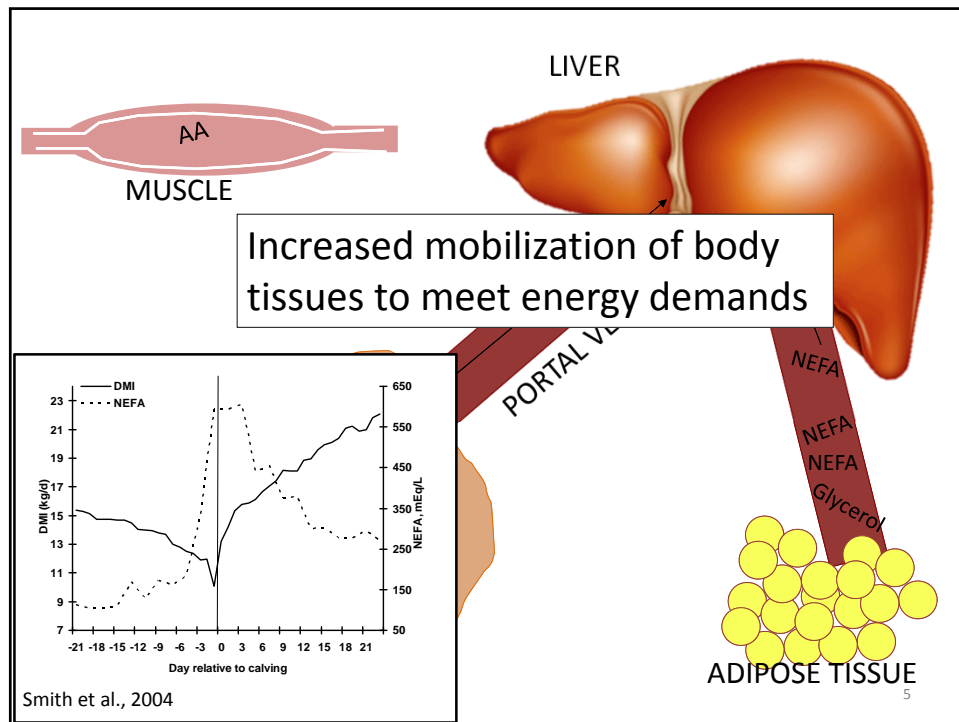
3

Comparison of estimated demands for nutrients pre and post calving

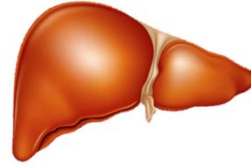


Bell, 1995

4



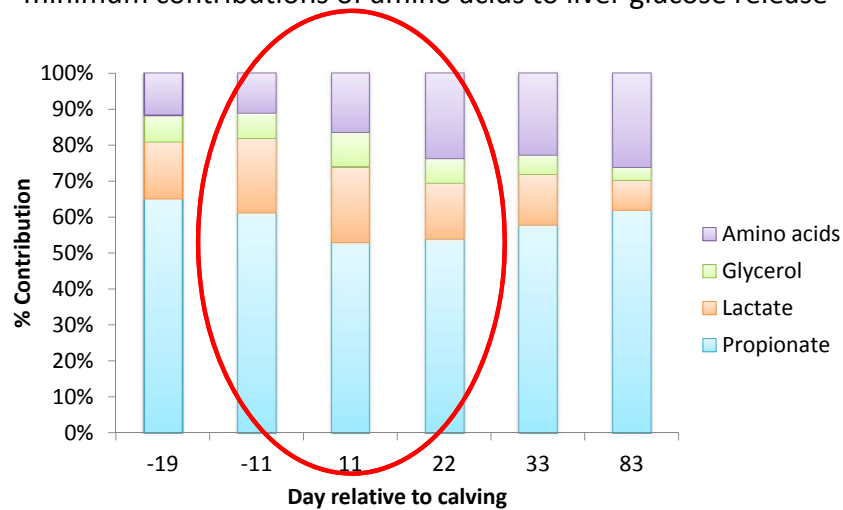
Glucose Production



- Gluconeogenesis
- Glucogenic Precursors:
 - Propionate
 - Lactate
 - Glycerol
 - Amino acids

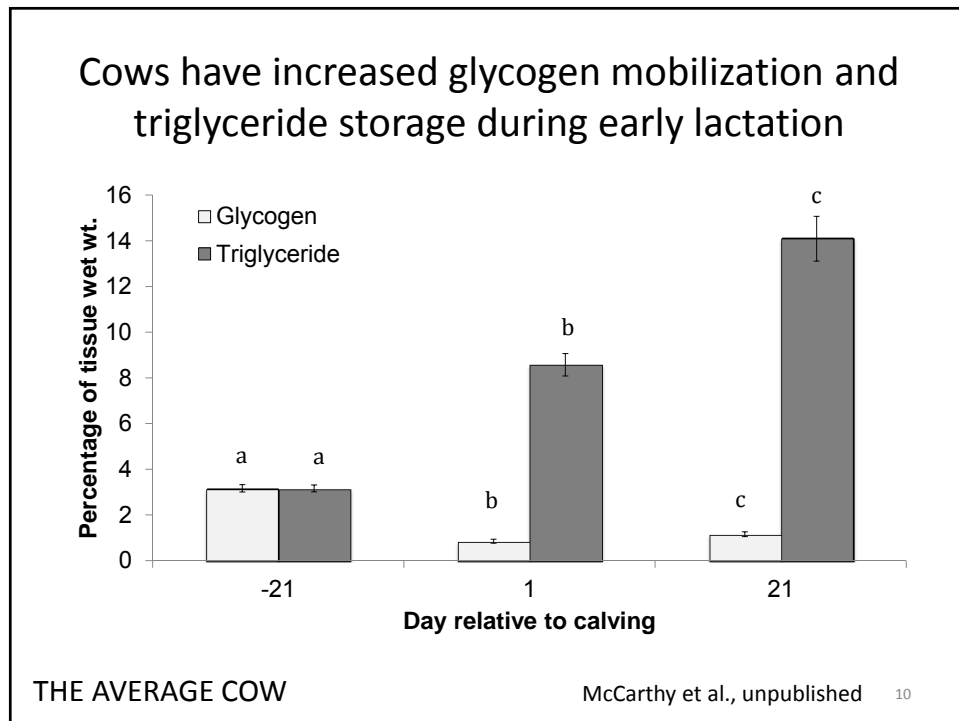
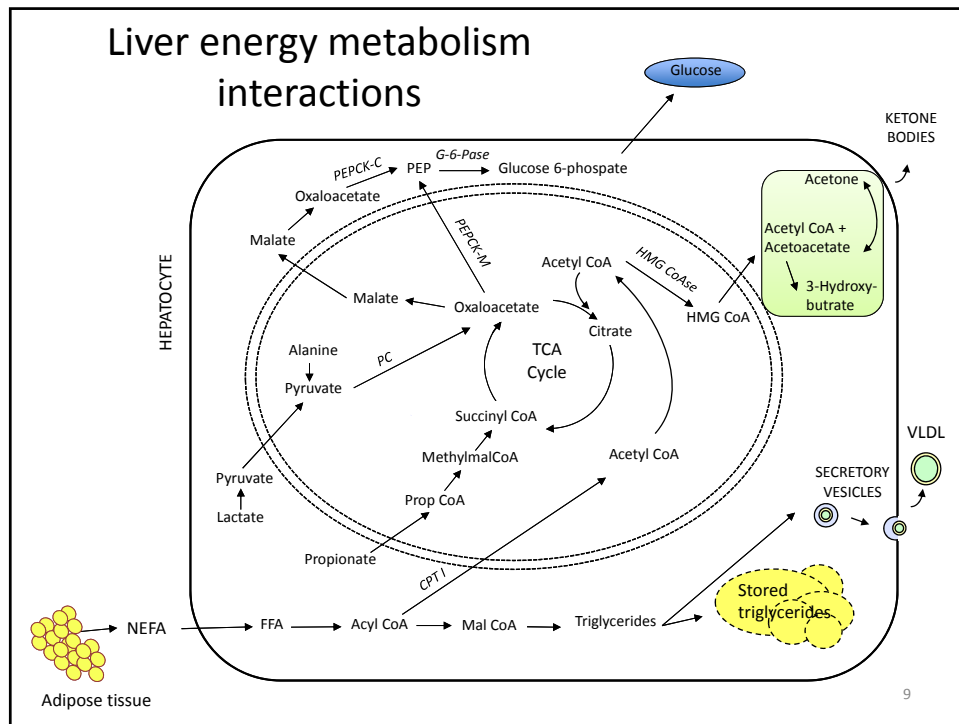
7

Maximum contributions of propionate, lactate, and glycerol and minimum contributions of amino acids to liver glucose release

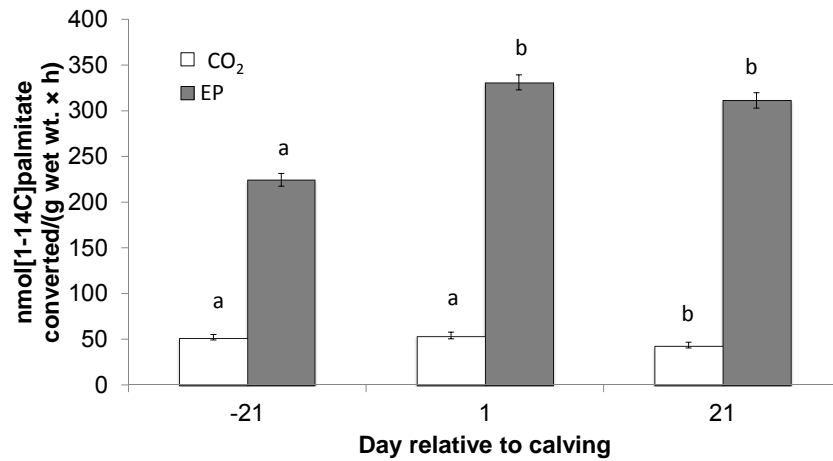


Reynolds et al., 2003

8



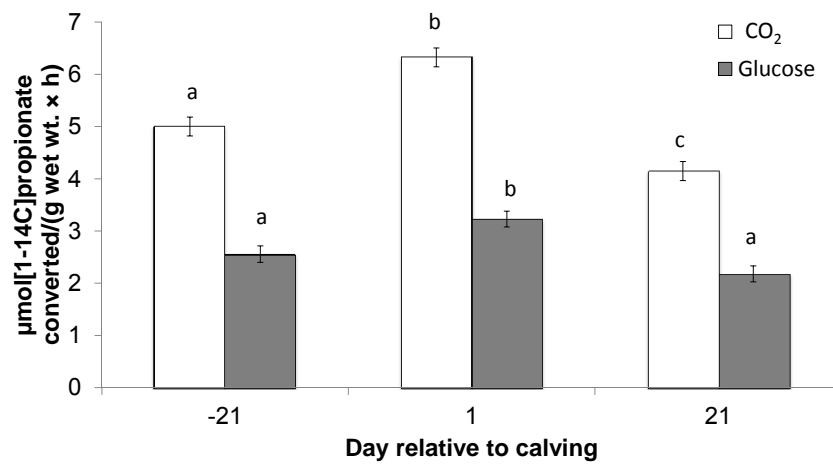
Increased in vitro reesterification of [1-¹⁴C]palmitate in early lactation liver slices



THE AVERAGE COW

McCarthy et al., unpublished 11

Increased in vitro oxidation and conversion of [1-¹⁴C]propionate to glucose in early lactation liver slices



THE AVERAGE COW

McCarthy et al., unpublished 12

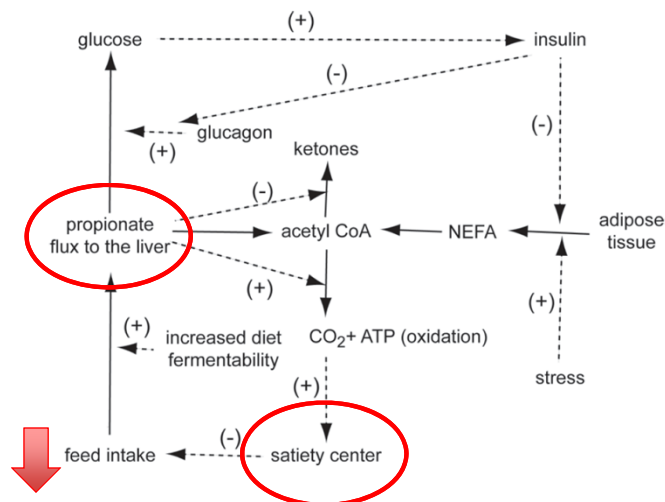
Strategies to Increase Propionate Production

1. Increase amount of dietary starch content
 - Higher starch
2. Increase starch fermentability
 - Processing (e.g. steam flaking)
3. Ionophores
 - Rumensin
 - Favor ruminal propionate production



13

Hepatic Oxidation



Allen et al., 2009

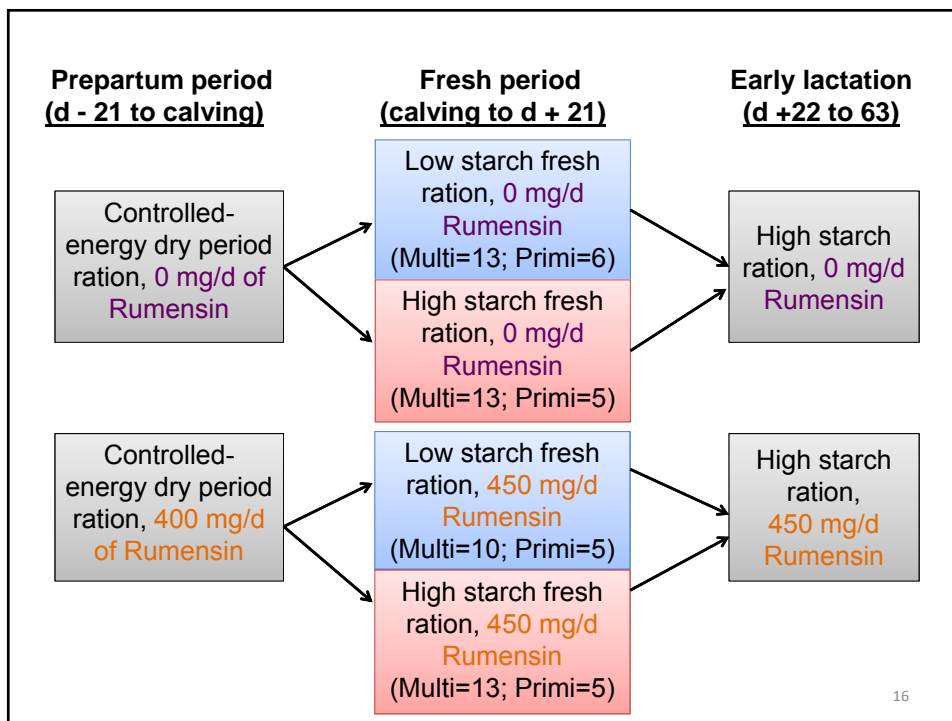
14

Liver Oxidation in Early Lactation

- Hypophagic effect of propionate on DMI probably less likely during early lactation:
 - Liver energy requirements increase at onset of lactation
 - NEFA likely are the main oxidative fuel for liver
- Liver may have the capacity to direct additional propionate toward glucose

Reynolds et al., 2003; Drackley et al., 2001

15

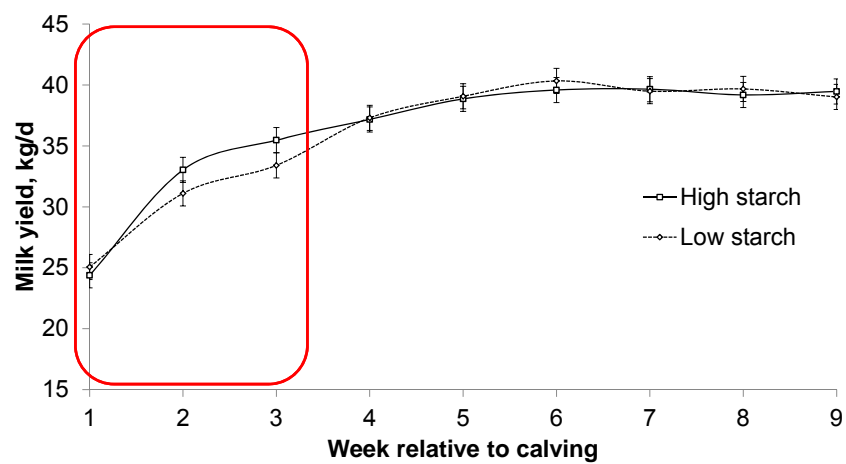


16

- No interactions of starch x rumensin

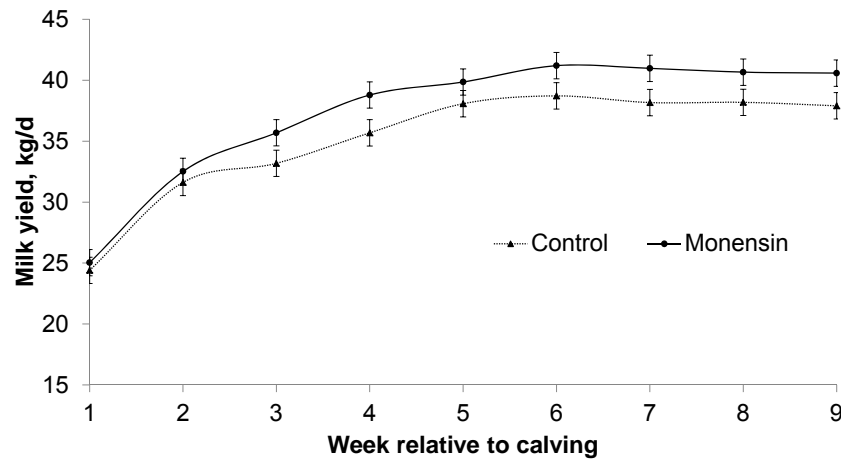
17

Higher starch → faster increase in milk



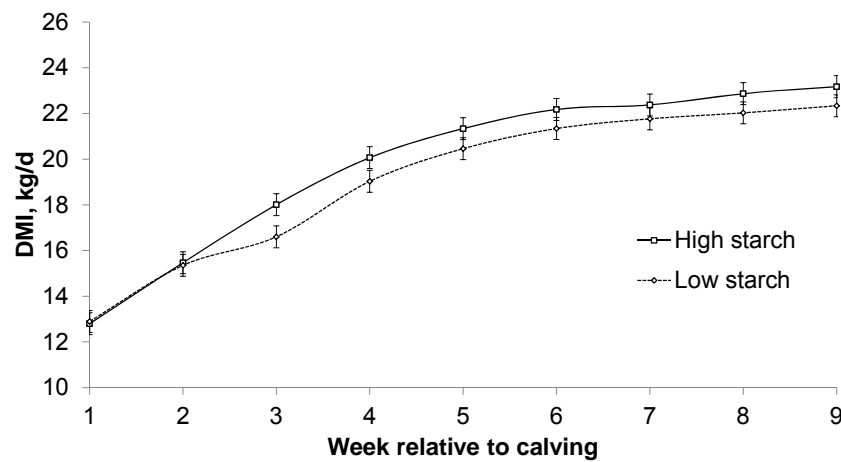
McCarthy et al., submitted 18

Transition period monensin → greater early lactation milk yield



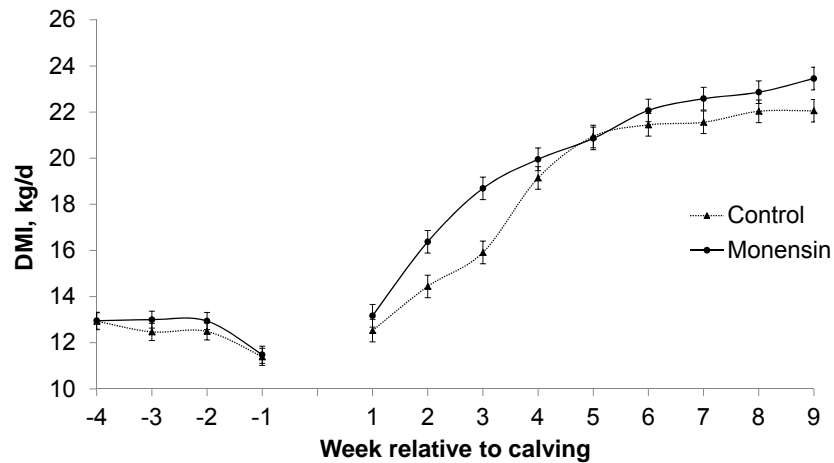
McCarthy et al., submitted 19

Higher starch → faster increase in DMI



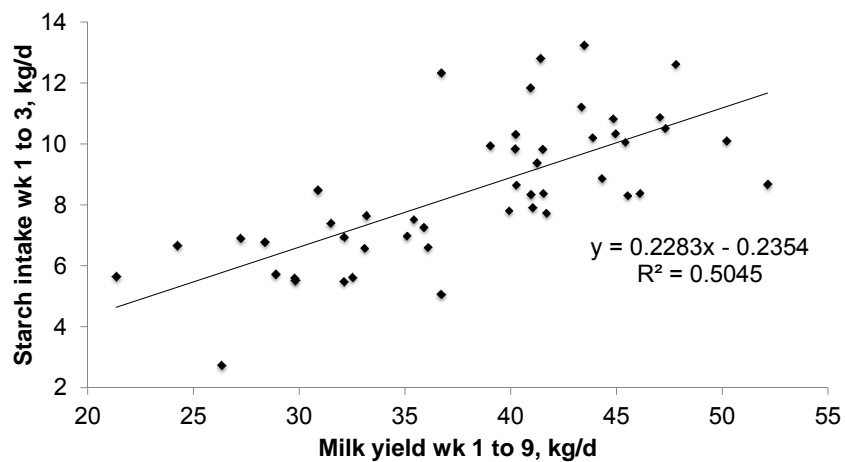
McCarthy et al., submitted 20

Transition period monensin → faster increase in post calving DMI



McCarthy et al., submitted 21

Greater early lactation starch intake → sustained increases in milk yield



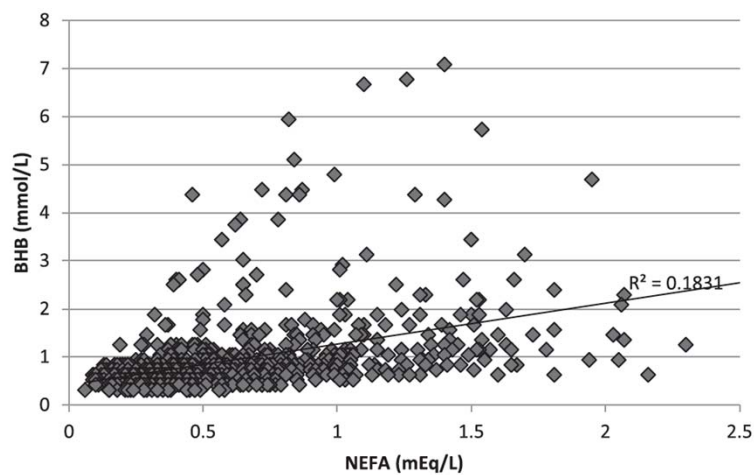
McCarthy et al., submitted 22

Markers of negative energy balance

- NEFA
- BHBA
- Elevation of both transition period BHBA and NEFA associated with negative downstream productive outcomes

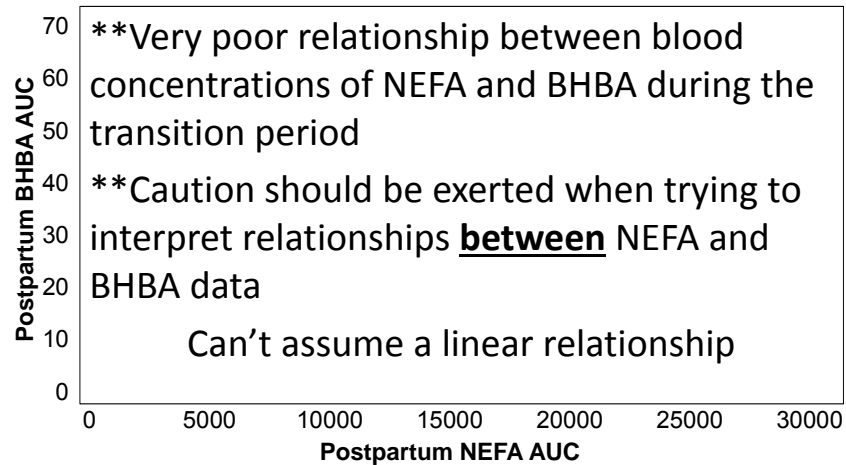
Ospina et al, 2013; McArt et al., 2013 23

Correlation between B-hydroxybutyrate (BHB) and Non-esterified fatty acids (NEFA) in cows sampled post-partum



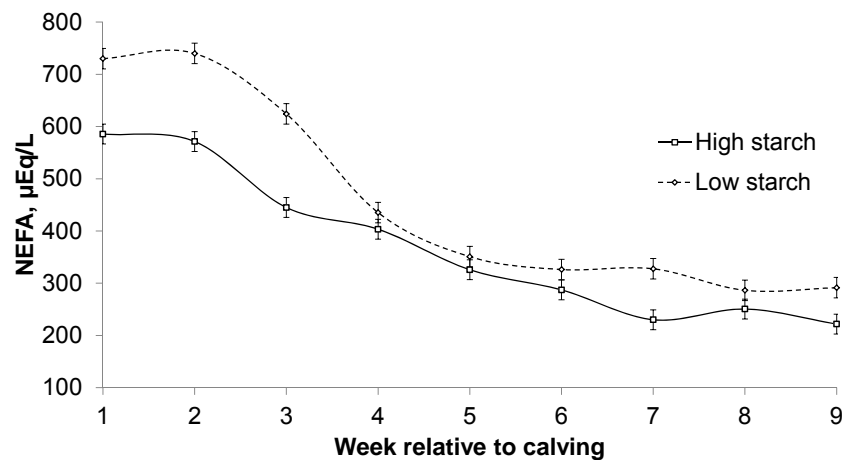
Ospina et al., 2013 24

Relationship between NEFA and BHBA



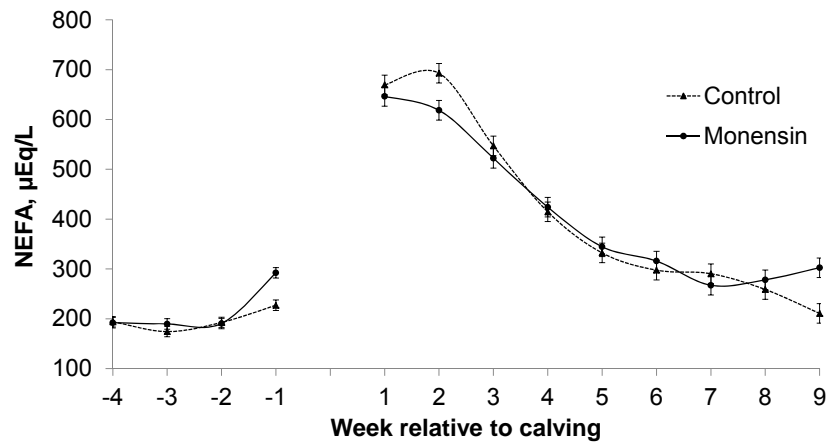
McCarthy et al., unpublished 25

Higher starch → less NEFA mobilization



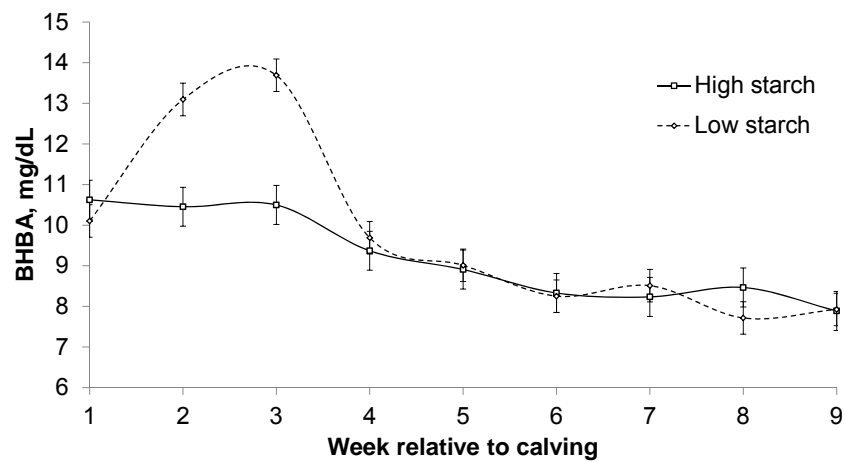
McCarthy et al., submitted 26

No effect of monensin on NEFA



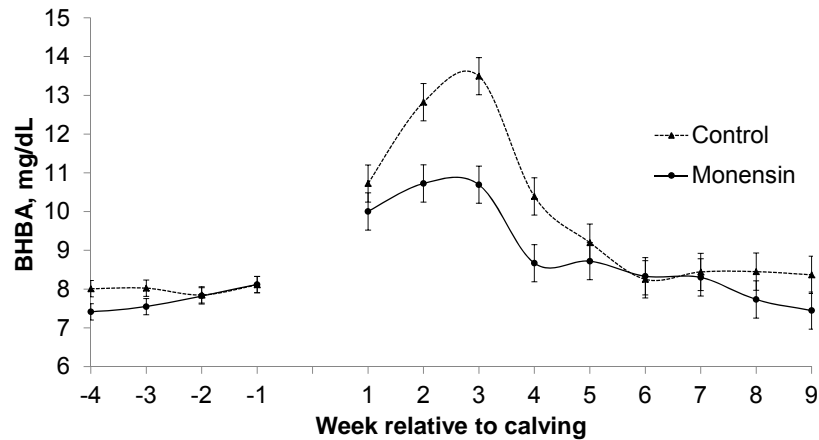
McCarthy et al., submitted 27

Higher starch → less BHBA



McCarthy et al., submitted 28

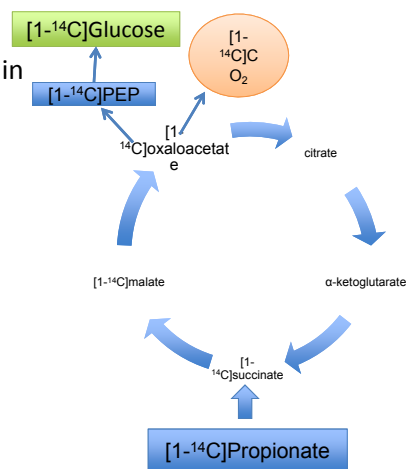
Transition period monensin → less BHBA



McCarthy et al., submitted 29

Ratio of glucose to CO₂

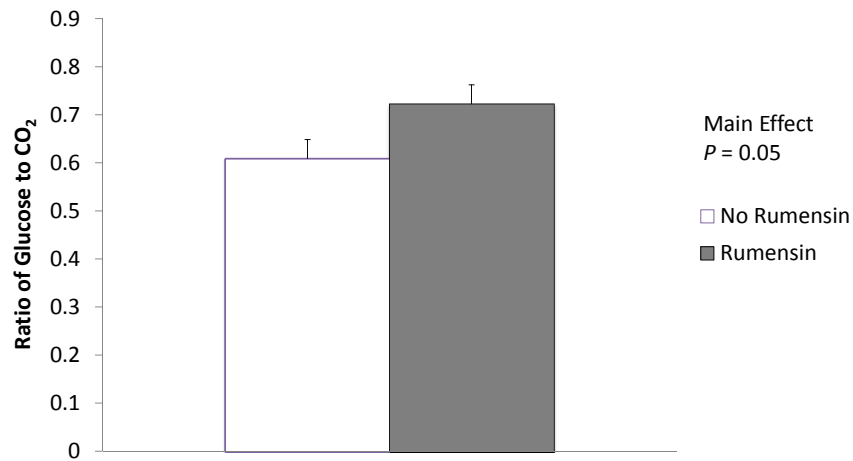
- [1-¹⁴C]propionate label randomizes in the TCA cycle
- 1 mole of propionate that form oxaloacetate should yield:
 - 0.5 moles radiolabeled glucose
 - 0.5 moles radiolabeled CO₂
- An increase in this ratio = greater efficiency in propionate utilization for glucose synthesis



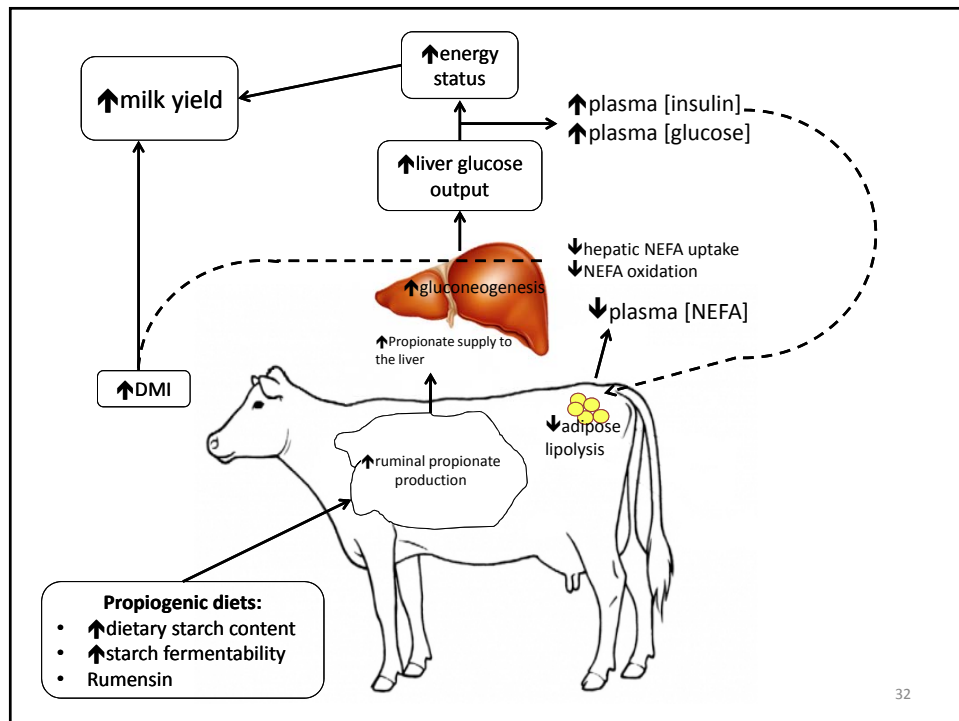
Knapp et al., 1992

30

Rumensin → increased ratio of glucose to CO₂



McCarthy et al., submitted 31



32

Implications

- Feeding a more propiogenic diet during early lactation:
 - Has a positive energetic impact
 - Increased milk production
 - Better metabolic profile
 - Increased propionate conversion to glucose
 - Does not negatively impact feed intake
 - Increased DMI
 - Less NEFA mobilization

33

Food for thought...

- peNDF content of your fresh cow ration?
- Ration formulated for BMR with 46% NEF and 26% starch
- New bunk of BMR 41% NDE and 34% starch

Item	Low starch	High starch
Cows, n	9	5
Retained placenta	2	1
Displaced abomasum	1	2
Clinical ketosis*	4	4

*BHBA > 2.5 mM on Precision Xtra meter.

34

peNEF in fresh cow rations?

- Adjusted ration by adding in more chopped straw
 - 6 lbs. DM vs. 2 lbs. DM previously
 - Decreased BMR by 4 lbs. DM
- Fresh cow DMI isn't likely limited by physical fill
- Increasing fiber content of the diet can improve intake via improved rumen health

35

Thank you!

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36

PHOSPHORUS NUTRITION OF DAIRY CATTLE – UPDATE

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Department of Animal Science
Cornell University

Phosphorus (P) continues to be of interest in dairy cattle rations due to both environmental and animal performance considerations. This topic was previously reviewed at this conference in 1999 (Satter, 1999; Chase, 1999). Since that report, the NRC has provided revised P requirements for dairy cattle (NRC, 2001). This paper will review some recent work in the area of P nutrition. In addition, preliminary results from a large, multi-state field trial using commercial dairy herds will be provided.

WHOLE FARM P BALANCE

Phosphorus is the mineral that is currently included as a component of comprehensive nutrient management plans on dairy farms. In some situations, nutrient management plans will be P based rather than nitrogen based. These regulations will require many dairy farms to reduce P excretion via manure to meet soil P application rates with their existing land base. A 5-year field study examined the possible shifts that could be made in P excretion in a commercial dairy herd (Tylutki et al., 2002). Total yearly manure P excretion was decreased from 43,435 lbs. to 31,192 lbs. over this time. During this same time period, the number of milking cows increased by 33% and total daily herd milk production increased by 45%. This change in excretion was the result of adjustments in ration P levels, feeding higher levels of home produced forages and purchasing less protein supplements for inclusion in the ration. The proportion of the total diet from home produced feeds increased from 43 to 59% over this same period.

A second study was conducted on 4 dairy farms in the Cannonsville Reservoir Basin to evaluate shifts in manure P levels and mass farm P balance (Cerosaletti et al., 2003). Dietary P adjustments were made in 2 of these herds. The initial farm P balances were 64 and 56%. Farm P balances were decreased to 38 and 46% after diet P was reduced by 25% in these herds. The total P remaining on these farms was reduced by 50%. It is projected that a 20% reduction in purchased feed P intake for all cows in this basin could lower feed P imports by 32-36% on a watershed basis (Cerosaletti et al., 2004).

Whole farm P balances have also been reported for 41 Western dairy farms (Spears et al., 2003). The average farm P balance was 37% on these farms. This is lower than farm balance values reported in many other studies. The primary reason was that either manure or compost was exported from these farms. The average P exported by this method was 28% of the total P imports. These farms were divided into those that grew some crops versus those that grew no crops. The farm P balance was 58% for the farms that grew no crops while it was 27% for those that grew crops. The farms that grew no crops exported an average of 40% of their total P imports as manure or compost.

REPRODUCTION

A study was conducted to evaluate the relationship between diet P content and estrus behavior in dairy cattle (Lopez et al., 2004a). Diet P levels of 0.38 or 0.47% were fed to cows for the first 34 weeks of lactation. A radio telemetric patch was placed on each cow at 40 days in milk (DIM). The information from this system was used to determine the length of the estrus cycle and the number and duration of the mounts. Visual estrus detection was also used. There were no differences in the duration of estrus, total mounts or total mounting time between the 2 levels of diet P used in this study.

A second trial was conducted using diet P levels of 0.37 and 0.57% P fed for the first 165 days of lactation (Lopez et al., 2004b). A radio telemetric patch was placed on all cows at 50 DIM. This device recorded mounting activity between days 50 to 100 of lactation. Visual estrus detection was also done in this study. There were no differences in days to 1st estrus, days to 1st service or first service conception rate in this study related to diet P level.

PHYTASE

A majority of the P present in cereal grains is typically organically bound as phytate P. Phytase is an enzyme that can release the phosphate groups from the phytate molecule. This increases the availability of the P to the animal. Phytase is commonly added to the diets of poultry and swine to increase the P absorbed from cereal grains and protein sources. In ruminants, hydrolysis of phytate P occurs in the rumen due to microbial activity (Yanke et al., 1998). Even though 100% of the phytate P may not be hydrolyzed in the rumen, phytase has not usually been added to ruminant diets.

A couple of recent papers have examined the potential use of added phytase in dairy cattle diets. One trial compared P digestibility in dairy cows fed no added phytase or added phytase (Kinciad et al., 2005). Total P digestibility was slightly higher for the diets containing added phytase. Fecal P concentration was also tended to be lower in the cows fed the added phytase diets. The authors of this study indicated that there may be some specific situations in which added phytase could be beneficial in dairy cattle diets. A second trial reported a tendency for increased apparent P digestibility when exogenous phytase was added to dairy cattle diets (Knowlton et al., 2005). P intake or excretion via milk, feces or urine was not different in this report.

Another trial examined the effect of added phytic acid on P use in dairy cattle (Guyton et al., 2003). Even though actual P intakes were higher in cows fed the added phytic acid, there was no difference in apparent P digestibility. Total daily P excretion was also higher for the cows fed added phytic acid. Additional information is needed before the addition of phytase or phytic acid to dairy cattle diets can be recommended.

P FIELD STUDY

A 3-year field study was conducted using commercial dairy herds to examine the relationship between diet P level, milk production, reproductive performance and fecal P levels. This was a multi-state (Pennsylvania, New York, Delaware, Maryland, Virginia) and multi-university (University of Pennsylvania Vet School, Cornell University, Penn State University, University of Maryland, University of Delaware and Virginia Tech). Commercial herds were selected for this study based on the levels of diet P being fed (Dou et al., 2003). Rations were formulated by the nutritional professional working with each herd. Monthly DHI was obtained for each herd over the 3 years of the study.

Table 1. Herd Descriptive Characteristics

Item	All Herds	New York Herds
Number of Herds	96	29
Cows/herd (average)	282	522
Cows/herd (range)	28 – 2370	47 – 2370
Herd milk, lbs/day (average)	72.3	76.6
Herd milk, lbs/day (range)	46.3 – 103.8	53.3 – 103.8
Conception rate, % *	33.5	33.7
Heat detection rate, % *	39.5	43.8
Pregnancy rate, % *	12.2	13.7
Days to 1 st Breeding *	86.9	82.9
Days open *	135.1	132.1

* Average

Table 2. Milk production and reproduction data in trial herds for the first 2 years of the study

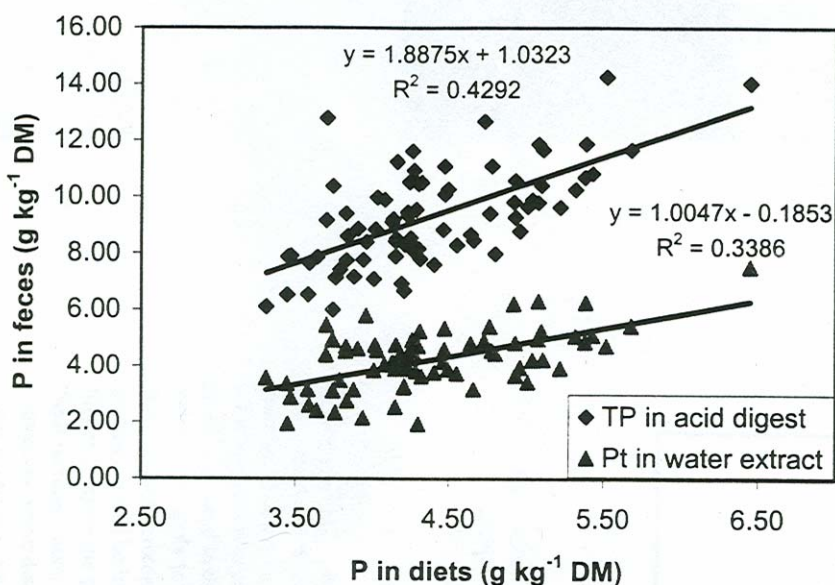
Item	Low Herds (<0.38% P)	Medium Herds (0.38 – 0.45% P)	High Herds (>0.45% P)
Number of Herds	18	56	22
Cows/herd	468	260	187
Average diet P, %	0.369	0.414	0.476
Milk, lbs/day	75.7	71.3	72.0
Conception rate, %	30.1	33.4	36.4
1 st Service Conception Rate, %	28.1	31.9	33.9
Heat Detection Rate, %	45.4	39.3	35.4
Days to 1 st Breeding	79.3	87.1	92.3
Days Open	129	137	135

Initial statistics have been done on the data for the first 2 years of this study. The key results of this analysis are:

- There were no significant differences in milk production, conception rate, pregnancy rate, 1st service conception rate or days open between the 3 diet P levels used in this field trial.
- There was a significant decrease in heat detection rate as diet P levels increased.
- There was a significant increase in days to 1st breeding as diet P levels increased.

Figure 1 contains information on the relationship of diet P levels and fecal P levels from this field study. Note that as diet P increases that fecal P also increases. This is true for both total P (TP) and the water soluble P (Pt). Other research papers have reported similar relationships of diet and fecal P levels (Bertrand et al., 1999; Toor et al., 2005).

Figure 1. Diet P and Fecal P



Ration and fecal P data were examined for 3 of the New York herds on the multi-state field study. One herd was selected from each of the diet P groups. The lowest fecal P value for an individual cow in this data was 0.42% on a DM basis. The highest individual cow had a fecal P content of 1.83%. Total yearly P excretion was calculated for each of these herds assuming 305 day lactation, daily fecal excretion of 100 lbs/day and a fecal DM content of 15%. The acres needed to spread the manure from each cow on a P basis were calculated assuming that only the crop removal rate (30 lbs/acre) of P could be applied. Table 3 contains the results of this analysis.

Table 3. Diet P, Fecal P Excretion and Acres/Cow Required for Manure Spreading

Item	Herd A	Herd B	Herd C
Diet P, % of DM	0.57	0.48	0.38
Total fecal P, % of DM	1.17	0.80	0.74
Water soluble fecal P, % of DM	0.54	0.44	0.35
Water soluble fecal P, % of total fecal P	46.6	55.8	46.9
Fecal P excretion, lbs/cow/year	53.4	36.5	33.9
Acres needed/cow	1.78	1.21	1.13
Acres needed, 100 milking cows	178	121	113

ACKNOWLEDGEMENTS

1. The 3-year field study was funded by USDA Initiative for Future Agriculture and Food Systems Grant No, 2001-52103-11334.
2. The principal investigators at the following institutions:
 - a. Dr. Z. Dou – University of Pennsylvania
 - b. Dr. R.A. Kohn – University of Maryland
 - c. Dr. Z. Wu – The Pennsylvania State University
 - d. Dr. K.F. Knowlton – Virginia Polytechnic Institute and State University
 - e. Dr. J. T. Sims – University of Delaware
3. The cooperating dairy producers and their feed professionals that cooperated in this study.

REFERENCES

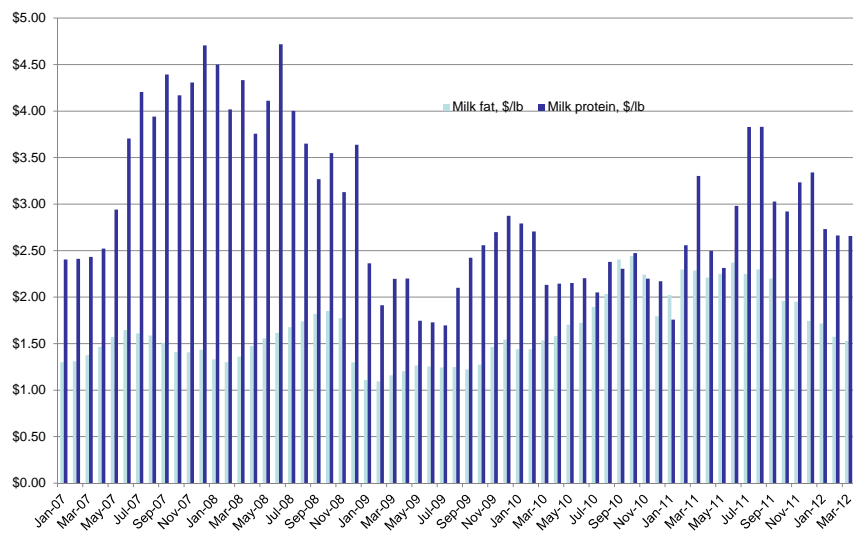
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Factors affecting milk components



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Cornell University



FMMO milk component values, 1/07 to 3/12

Many factors can affect milk fat

Nutritional Factors

Dietary CHO

Unsaturated fats

feeding strategy

ionophores

Milk fat

Non-nutritional Factors

genetics

stage of lactation

season

parity

ambient temperature

Many non-nutritional factors affect milk fat

- Genetics/breed
- Days in milk
- Season
- Heat stress
- Feeding patterns/stocking density
- Sampling strategy/analytical methods

Many non-nutritional factors affect milk fat

- Genetics/breed
- Days in milk
- Season
- Heat stress
- Feeding patterns/stocking density
- Sampling strategy/analytical methods

Table 3. Heritability (h^2) estimates for milk and its components.

Trait	Holstein		Jersey	
	h^2	SD ¹	h^2	SD ¹
Fat, %	0.58	0.23	0.55	0.28
Protein, %	0.51	0.14	0.55	0.20
Fat, lb	0.30	52	0.35	50
Protein, lb	0.30	37	0.35	36
Milk, lb	0.30	1444	0.35	1204

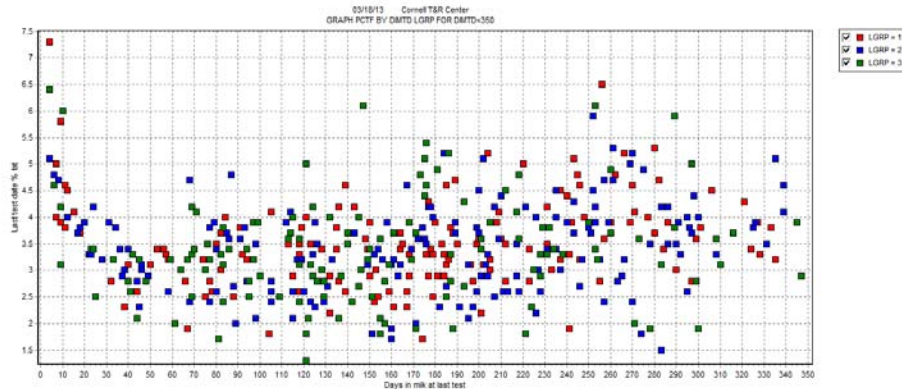
¹Estimate of genetic standard deviation.

Source: USDA-AIPL yield traits definition (May 2005) and trend estimates for cows born in 2000.

Source: Heinrichs et al., 2005

Many non-nutritional factors affect milk fat

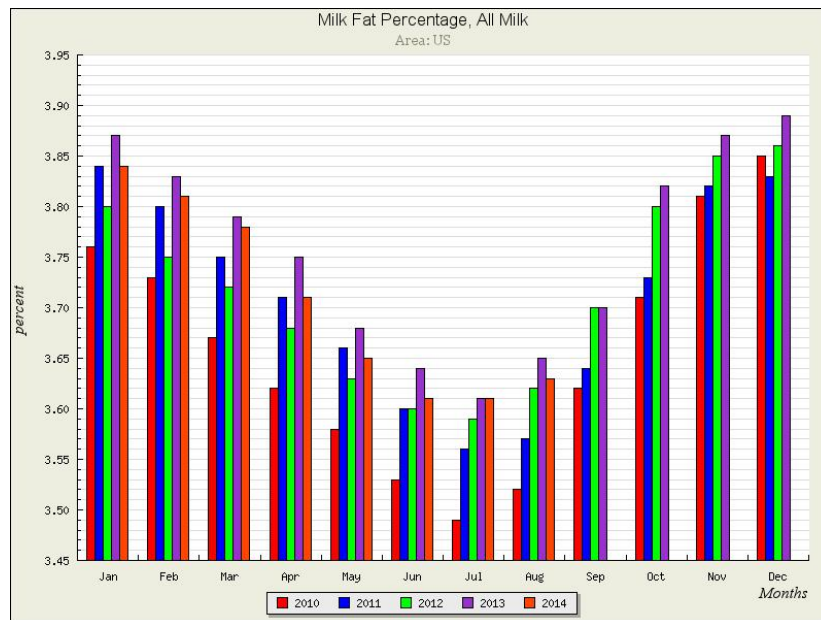
- Genetics/breed
- Days in milk
- Season
- Heat stress
- Feeding patterns/stocking density
- Sampling strategy/analytical methods



Milk fat percentage by days in milk (test day snapshot from Cornell T&R Center)

Many non-nutritional factors affect milk fat

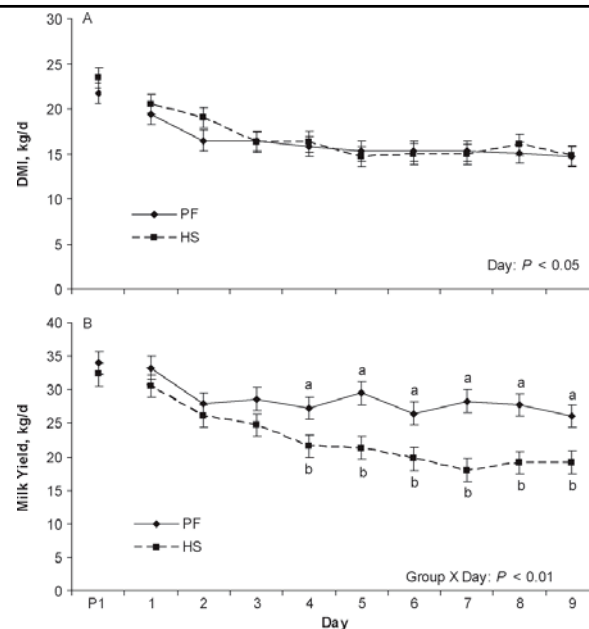
- Genetics/breed
- Days in milk
- Season
- Heat stress
- Feeding patterns/stocking density
- Sampling strategy/analytical methods



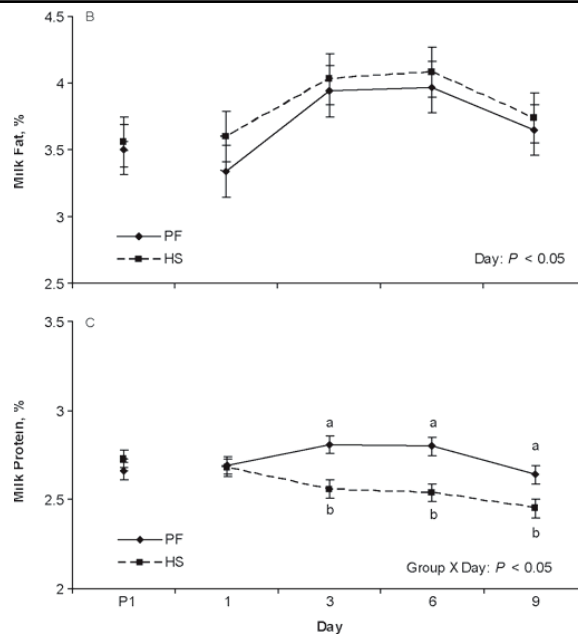
Source: http://future.aae.wisc.edu/data/monthly_values/by_area/450?area=US&tab=production&yoy=true

Possible explanations for seasonality in milk fat percentage

- Changes in silage quality/characteristics?
- Photoperiod?
 - Prepartum day length negatively correlated with milk yield and milk fat and protein percentage (Aharoni et al., 2000)
- Changes in feeding behavior?
- Heat stress



Effects of heat stress (HS) and pair-feeding (PF) on DMI and milk yield. From Rhoads et al., 2009)



Effects of heat stress (HS) and pair-feeding (PF) on milk fat and protein percentages. From Rhoads et al., 2009)

Many non-nutritional factors affect milk fat

- Genetics/breed
- Days in milk
- Season
- Heat stress
- Feeding patterns/stocking density
- Sampling strategy/analytical methods

Intake, Milk Yield, and Milk Composition by Stocking Rate (Miner Institute)

Item	Stocking Rate, %				SE	P - value
	100	113	131	142		
DMI ¹ , kg/d	24.4	24.8	25.0	25.3	0.65	0.69
Milk, kg/d	41.4	40.7	41.5	41.1	0.32	0.39
Fat, %	3.84^a	3.77^{ab}	3.77^{ab}	3.67^b	0.05	0.03
Protein, %	3.05	3.03	3.03	3.03	0.02	0.66
Lactose, %	4.89	4.88	4.90	4.90	0.01	0.42
SCS ²	3.2	3.1	3.4	3.6	0.39	0.62

¹ DIM = Dry matter intake

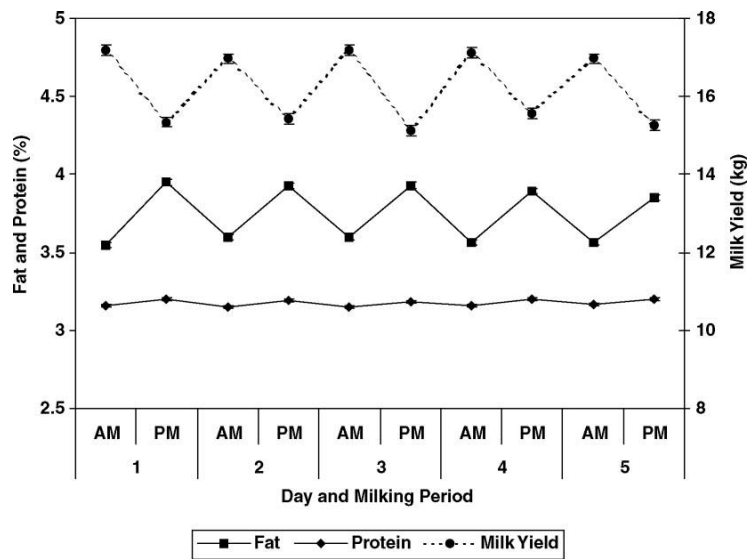
² SCS = Somatic cell score

^{a,b} Means within rows with different superscripts differ ($P < 0.05$)

Many non-nutritional factors affect milk fat

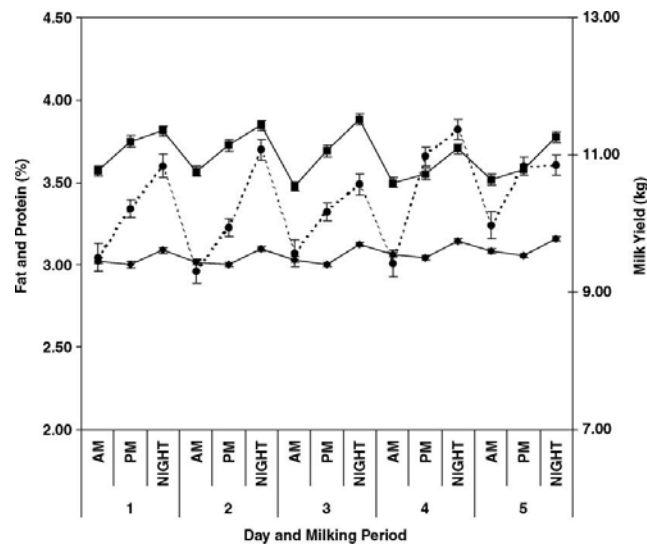
- Genetics/breed
- Days in milk
- Season
- Heat stress
- Feeding patterns/stocking density
- Sampling strategy/analytical methods

Variation in milk yield and milk fat and protein content by milking for herds milking 2X



Quist et al., 2008. J. Dairy Sci. 91:3412–3423

Variation in milk yield and milk fat and protein content by milking for herds milking 3X



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Summary opinion – these are responsible for variation in milk fat within a herd over time and among herds, but rarely, if ever are they the cause for low milk fat on farms

Many factors can affect milk fat

Nutritional Factors

Dietary CHO
Unsaturated fats
feeding strategy
ionophores

Milk fat

Non-nutritional Factors

genetics
stage of lactation
season
parity
ambient temperature

Dietary components can impact the risk of MFD in 3 ways

1. Increase C18 PUFA Precursors

Linoleic acid
(*cis*-9, *cis*-12 18:2)



Rumenic acid
(*cis*-9, *trans*-11 CLA)



Vaccenic acid
(*trans*-11 18:1)



Stearic acid
(18:0)

2. Alter BH pathways/rumen environment

trans-10, *cis*-12 CLA



trans-10 18:1



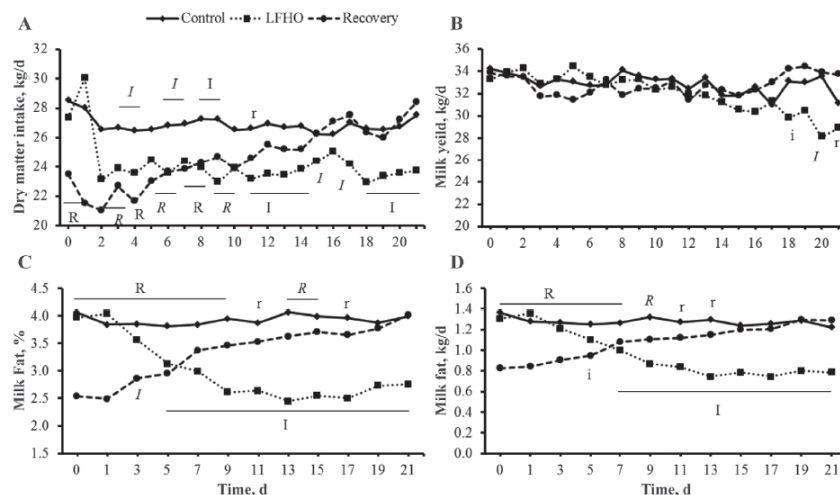
Stearic acid
(18:0)

3. Inhibit final step/ alter rates of BH

Summary -- common observations for low milk fat

- Factors that cause altered ruminal biohydrogenation
 - NDF and NFC interrelationships
 - Altered corn silage fermentation profiles?
 - Mycotoxins in forages or high moisture corn?
 - Elevated mold/yeast counts in high-moisture corn or silages?
 - Oxidized components of feedstuffs?
- Factors that result in high availability of linoleic acid
 - Unsaturated fat source, amount, and processing
- Factors that slow rates of biohydrogenation
 - Fish fatty acids
 - Ionophores
 - High C18:1 intake?
- Factors that result in high rates of passage
 - High production/DMI
- Most often not one factor, but an INTERACTION AMONG SEVERAL FACTORS, responsible for milk fat problems

Time courses during induction and recovery from milk fat depression



Rico and Harvatine, 2013. J. Dairy Sci. 96 :6621–6630

Dr. Dave Barbano research (2014 ADSA-ASAS JAM)

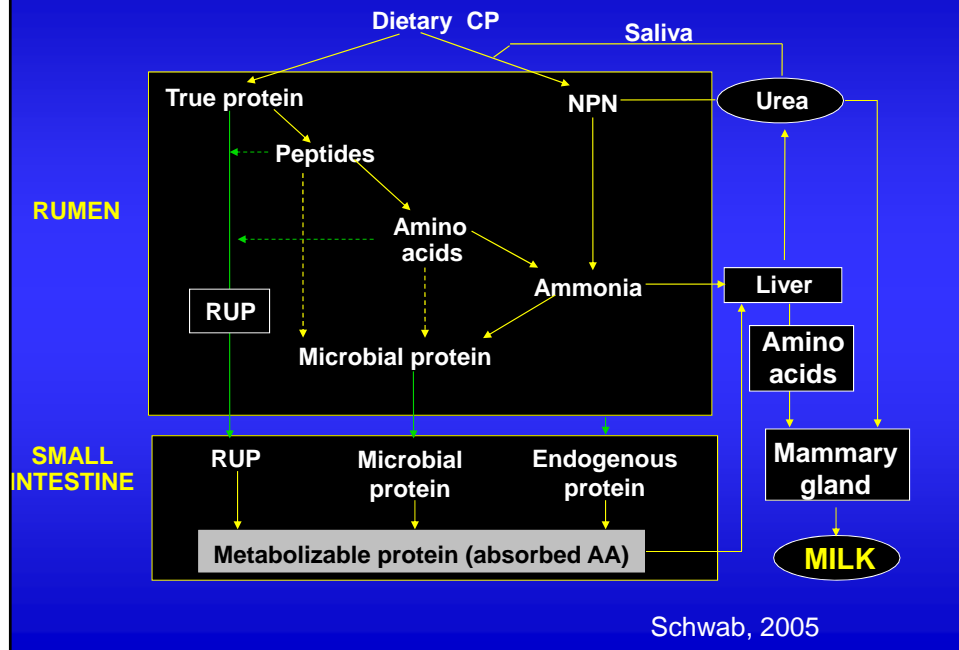
- Large field study (430 farms in Northern VT and Northern NY)
 - Sampled multiple times per month for 14 mo
 - Tested for fat, protein, lactose, and FA composition by mid-infrared analysis
- Key results
 - Wide variation in FA composition among herds
 - De novo FA content strongly and positively correlated with overall milk fat and protein percentages
 - Mixed and preformed also correlated positively with components, but not as strongly as de novo FA content
 - Total unsaturation negatively correlated with both overall fat and protein content of milk

Barbano et al., 2014. J. Dairy Sci. 97(E. Suppl. 1):320

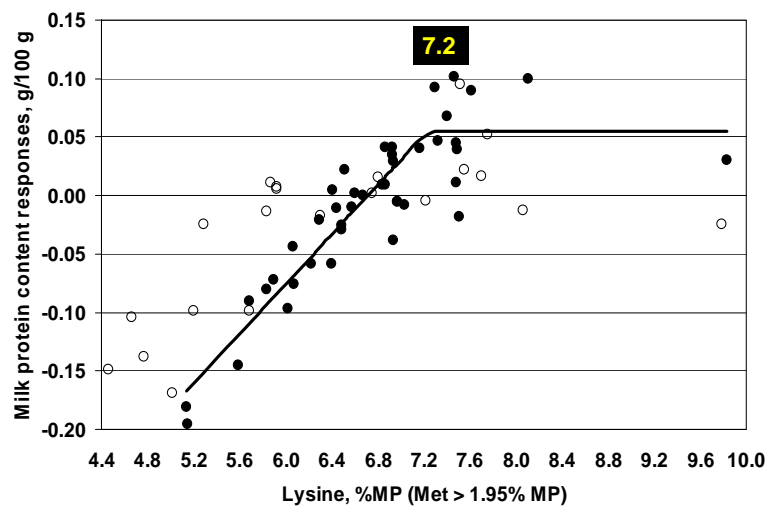
Factors that increase milk protein yield

- Nutritional/managerial factors that increase milk yield
 - Milking frequency
 - Forage quality
 - Cow health
 - Environmental factors (facilities, comfort, heat abatement, etc.)
- Shortened dry period length?
- Ration formulation approaches that specifically increase milk protein

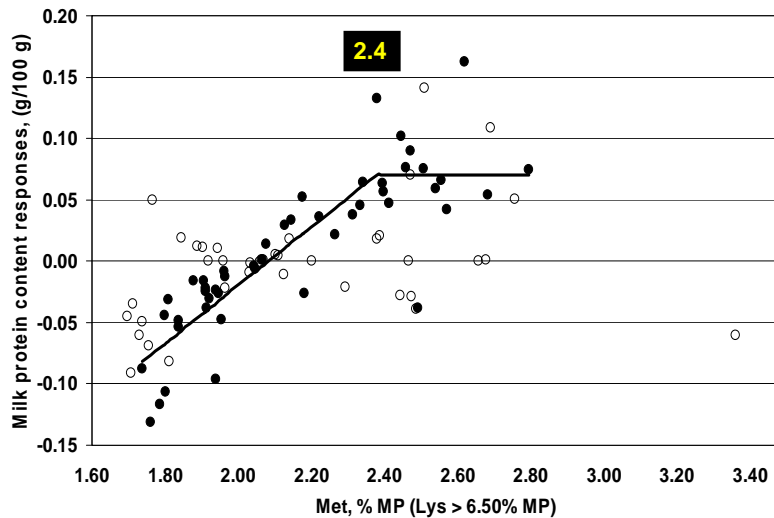
Protein metabolism in cows



Lysine Plot (NRC, 2001)



Methionine Plot (NRC, 2001)



Commercial Rumen Protected Methionine (RPM): Meta-Analysis

- Studies
 - 17 for Mepron
 - 17 for Smartamine
 - 1 Study for both
- 75 diet comparisons
 - 1040 individual cows
- Average of 20 g RP-Met/d
 - 12 g metabolizable Met

Courtesy Dr. Sarah Boucher

Patton R.A., 2010

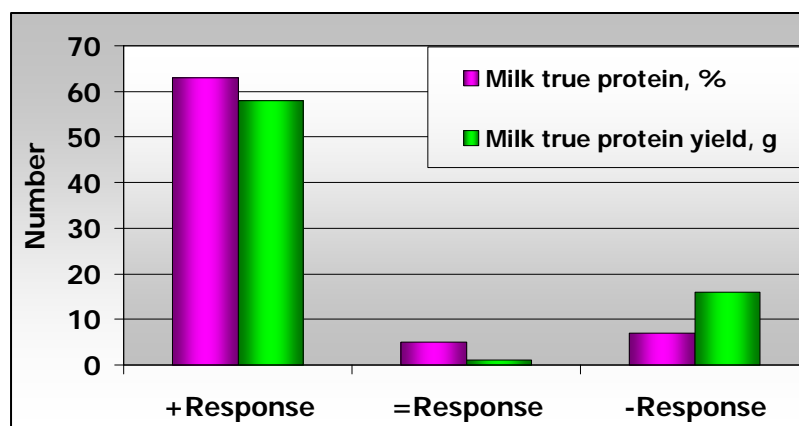
Patton, 2010: Meta-Analysis

Item	Mean	Min.	Max.
DMI, kg	-0.04	-2.10	1.50
Milk, kg	0.02	-4.20	4.40
Milk true protein, %	0.07	-0.09	0.35
Milk true protein, kg	0.03	-0.07	0.19
Milk fat, %	-0.01	-0.30	0.41
Milk fat, kg	0.01	-0.19	0.19

Courtesy Dr. Sarah Boucher

Patton, R.A., 2010

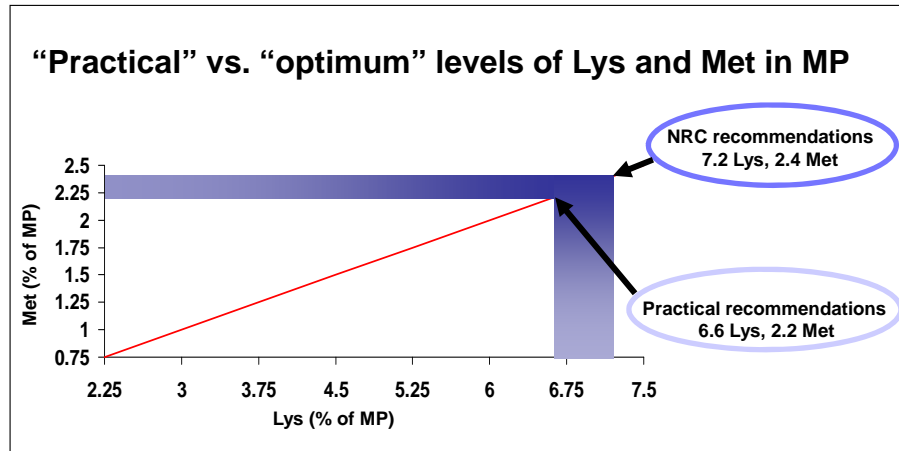
Meta-Analysis: Responses to RP-Met



Courtesy Dr. Sarah Boucher

Patton, R.A., 2010

“Optimum” vs. “practical” levels of Lys and Met in MP



Courtesy: Dr. Chuck Schwab

Why variability in response to AA balancing approaches?

- Lots of reasons related to ability to predict/model responses to AA balancing
 - Other limiting AA?
 - Accuracy of both MP and individual AA predictions
 - Digestibility/availability of protein/AA sources
 - Facility/behavioral factors that affect ruminal metabolism of rations
 - Management factors on individual dairies
 - Variation in optimal ratios at different stages of lactation

AA supply probably does not fully explain responses

- Doepel et al. (2004) suggested decreasing efficiency of MP use for milk protein as MP supply increases
- Relatively low (~20%) efficiency of use of abomasally infused casein for milk protein synthesis (summarized by Griinari, 1996)

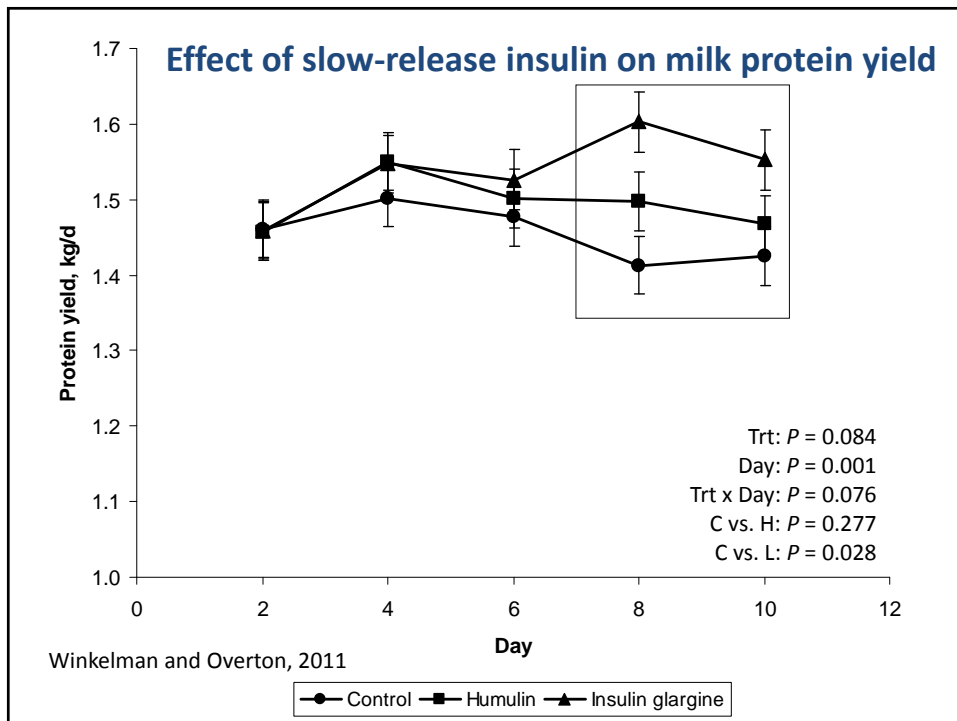
Role of energy nutrition in milk protein synthesis

- Sporndly (1989) reported much stronger relationship of milk protein percentage with dietary energy intake than dietary protein intake
 - Often attributed to ruminal fermentation and microbial protein synthesis

Table 1. Ingredient and nutrient composition (% of DM unless otherwise noted) of basal diet

Item	Value
Ingredient	
Corn silage	46.8
Shelled corn, finely ground	15.5
Wheat straw	6.90
Corn germ meal	5.22
Corn distillers grains	5.18
Canola meal	5.14
Amino Plus ¹	4.69
Mineral and vitamin mix ²	2.97
Soybean meal	1.71
Blood meal	1.64
Citrus pulp, dry	1.60
Energy Booster ³	1.10
Molasses	0.70
AminoShure-L ⁴	0.50
Urea	0.34
Alimet ⁵	0.08
Smartamine-M ⁶	0.08
Energy and nutrients ⁷	
NE _L , Mcal/kg	1.67
NDF	34.8
NFC	42.3
Starch	30.5
Ether extract	3.8
ME allowable milk, ⁸ kg/d	47.7
MP allowable milk, ⁸ kg/d	49.3
Lys, ⁸ % of MP	7.33
Met, ⁸ % of MP	2.54
CP	15.2
Lignin	3.4
Ca	0.59
P	0.39
K	1.15
Mg	0.27

Basal diet composition;
Winkelman and Overton, 2013;
J. Dairy Sci. 96:7565–7577



Summary and speculation

- Nonnutritional factors can vary milk fat on farms
 - Probably more responsible for variation and/or modest reductions in milk fat
 - Dietary factors likely most responsible for acute decreases in milk fat
- Recent data support 10 d to 2 wk timecourse for resolution of diet induced milk fat depression
- Milk protein can be modulated on dairies
 - Role of MP/AA
 - Role of energy?
 - Value of lower CP/more balanced overall delivery of AA?

Shredlage – What Have We Learned?

Dr. L. E. Chase
Department of Animal Science
Cornell University, Ithaca NY

Dairy producers are growing more corn silage and using higher levels of corn silage in dairy rations. The goal is to be able to make the best use of corn silage in the ration. A number of changes have occurred that have improved the nutritive value of corn silage. These include better hybrid genetics, selection of hybrids for fiber sand/or starch digestibility, kernel processing and more attention to harvesting dry matter and silo management. A new processing technique called shredlage was introduced about 3 years ago. This process rips or tears the corn stalk into longer pieces. The process also calls for setting the processing rolls tighter to smash the corn kernels. The TLC (theoretical length of cut) is recommended to be set at 26-30 mm foot corn silage with a moisture content of 65 to 70%. The suggested guideline for the processing rolls is 1.75 to 2.25 mm. As the corn silage gets drier, the TLC is reduced to 21 to 23 mm and the processing rolls are set at 1.5 to 1.75 mm.

At the 2014 Empire Farm Days seminar on shredlage, Michelle Woodman from Landmark Services Cooperative in Wisconsin provided some data on the change in particle size distribution using the Penn State shaker box. Table 1 contains these results. The more coarsely harvested shredlage has a higher proportion of longer particles on the top screen compared to a shorter TLC or conventional KP processing. However, the total on the top 2 screens is similar for the 3 shredlage results and slightly higher than the KP harvested corn silage. This could indicate a higher peNDF (physically effective NDF) value for shredlage assuming no sorting against long particles when fed to cows. Figure 1 is an example of particle size of shredlage harvested at a 30 mm TLC.

Table 1. Corn Silage Particle Size Distribution^a

Shredlage	% on Top Screen	% on Screen 2	% on Screen 3	% in the Pan
30 mm TLC	35	45	19	1
22 mm TLC	18	58	22	2
17 mm TLC	9	71	18	2
Conventional KP	8	60	30	2

^a Source: Michelle Woodman, 2014

The information to date indicates that harvesting corn silage as shredlage is a slightly slower process, requires more power and takes more fuel than harvesting using a KP unit. Reports indicate that custom harvesters may be charging \$1-2/ton more when harvesting shredlage to account for these differences.

There is also a concern with the bunk silo packing density when using shredlage. At the EFD seminar, Corwin Holtz from Holtz-Nelson Consultants indicated he is seeing slightly higher packing densities (1-2 lbs. DM/cubic foot) on farms using shredlage. At Cornell, our silo densities have been similar for both shredlage and KP corn silages. Other reports indicate that corn silage harvested as shredlage is at least equal in packing density compared with silage harvested using KP.

There have been 2 research trials conducted using shredlage at the University of Wisconsin-Madison by Dr. Randy Shaver. In the first trial, rations contained 50% corn silage, 10% alfalfa silage and 40% concentrate on a dry matter basis. The only difference between the 2 rations was the source of corn silage. Rations were fed for an 8 week period. Cows fed the shredlage ration tended to consume more dry matter and higher 3.5% fat corrected milk. The difference in milk was 2.2 lbs. higher for cows fed shredlage. The difference in milk production between the rations increased the longer the shredlage ration was fed. Total tract starch digestibility and NDF digestibility were also higher in cows fed the shredlage ration. A second trial was conducted using BMR corn silage harvested as shredlage or KP. Results from this trial are not yet available. We have a trial in progress at Cornell. The rations contain 50% corn silage, 14% alfalfa silage and 36% concentrate on a dry matter basis. The processing method for corn silage is the only difference in the 2 rations. The shredlage and KP silages were harvested at the same time in the same fields using 2 forage harvesters. One had a shredlage head while the other was KP. This trial should be completed at the end of September.

Comments from dairy producers using shredlage have indicated that they have been able to lower or eliminate the amount of dry hay, whole cottonseed or straw in dairy rations. In some cases, they have also reduced some of the corn grain fed due to the higher starch digestibility in the shredlage. At the EFD seminar, Corwin Holtz presented information based on a herd in Wisconsin using shredlage. The assumptions used were feeding 1 lb. less corn grain, replacing 1 lb. of dry matter from haylage with 1 lb. of dry matter from shredlage and increasing milk production by 1 lb. per cow. In this example, there was an increase in income of 28.5 cents/cow/day. It will be useful to do similar calculations in additional herds.

At this point, the information on shredlage looks promising in terms of increasing the nutritive value of corn silage. However, it must be remembered that the corn silage processing score (CSPS) and starch digestibility of shredlage has been higher than the KP samples. Results may have been different if the KP silages had a higher corn silage processing score. Shredlage offers an opportunity to adjust rations by removing some (or all) of the dry hay and straw used in some rations. It also provides an opportunity to provide more rumen and total tract starch digestibility which could result in feeding less corn grain. Additional data is still needed on potential differences in peNDF, NDF and starch digestibility. Initial reports and experiences have

been positive and encouraging. Additional research and experiences over the next couple of years are needed to provide additional information for decision making. If you don't have an option to harvest your corn silage as shredlage, make sure that you do the best job possible with kernel processing. There are still too many KP samples with low CSPA scores. Adjusting the rolls to better process the kernels in your current harvester may be a quick way to improve starch digestibility in your current situation.

